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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, 16/28, C12Q 1/68, G01N 33/68 — A2

(11) International Publication Number: WO 95/33048

(43) International Publication Date: 7 December 1995 (07.12.95)

(21) International Application Number: PCT/EP95/01968

(22) International Filing Date: 24 May 1995 (24.05.95)

(30) Priority Data:

9410664.8	27 May 1994 (27.05.94)	GB
9502480.8	9 February 1995 (09.02.95)	GB

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: P_{2X} RECEPTORS (PURINOCEPTOR FAMILY)

(57) Abstract

The P_{2X} receptor of ATP has been cloned and expressed by recombinant DNA technology, so the receptor can be prepared free from other ATP receptors. The P_{2X} receptor enables antibodies to be prepared and is useful in screening compounds for use in a variety of diseases and conditions, including epilepsy, cognition, emesis, pain (especially migraine), asthma, peripheral vascular disease, hypertension, diseases of the immune system, irritable bowel syndrome and premature ejaculation.

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P2x RECEPTORS (PURINOCEPTOR FAMILY)

This invention relates to the P_{2X}-purinoceptor, its preparation and uses.

The P_{2X}-purinoceptor is a ligand-gated ion channel; that is, the receptor itself forms an ion channel which opens when extracellular adenosine 5'-triphosphate (ATP) binds to the receptor. There are five other classes of neurotransmitter receptors (nicotinic acetylcholine, glutamate, glycine, $GABA_A$ and $5\text{-}HT_3$); these form a structurally related superfamily of ligand-gated ion channels (Barnard, *Trends Biochem. Sci.* 17, 368-374, (1992)). The P_{2X}-receptor now identifies a new family of this type of receptor. The unique structure of this receptor, the widespread distribution of this receptor throughout the body, and the numerous physiological roles this receptor may play, make it an important protein that can be used to identify new, therapeutically effective, compounds for the treatment of a number of pathological states.

In 1929 the eminent physiologist Szent-Gyorgyi described powerful cardiovascular actions of extracellular purine nucleosides (e.g. adenosine) and nucleotides (e.g. ATP) (Drury & Szent-Gyorgyi, *J. Physiol.* 68 213-237 (1929)), but it was not until 1972 that pharmacological evidence was provided to suggest the existence of distinct receptors for extracellular ATP (ie. that recognise ATP but not adenosine) (Burnstock, *Pharmacological Reviews* 21 509-581 (1972)). The seminal and subsequent work on this area by Burnstock and colleagues was largely unaccepted throughout the 1970s and early 1980s until the development of a range of relatively selective ligands

and techniques for directly measuring ATP release overwhelmingly substantiated Burnstock's hypothesis (Barnard *et al.*, *Trends Pharmacol. Sci.* 15 67-70 (1994)). In the past four or five years, unequivocal evidence for the role of ATP as a neurotransmitter has been provided for sympathetic control of blood flow to the intestine and smooth muscle tone (contractility) in genitourinary tissue such as vas deferens, bladder and ureter (Barnard *et al.* (*loc. cit.*) and Evans & Surprenant, *Brit. J. Pharmacol.* 106 242-249 (1992)). Substantial indirect evidence also exists for the role of ATP as a neurotransmitter in a number of distinct neurones in the spinal cord, autonomic ganglia and certain nuclei in the central nervous system (Bean, *Trends Pharmacol. Sci.* 15 67-70 (1992), Evans *et al.*, *Nature* 357, 503-505 (1992) and Edwards *et al.*, *Nature* 359 144-147 (1992)).

Purinoceptors are classified as P_1 (adenosine as ligand) and P_2 (ATP as ligand). The P_2 receptors are subclassified into two broad types - those that are 7-transmembrane receptors that couple to G-proteins (P_{2Y}, P_{2U}, P_{2T}, and perhaps P_{2Z}) and those that form a directly gated ion channel (P_{2X}). Pharmacological and/or physiological evidence for subtypes of each of these types of receptors exists. The most recent nomenclature for these receptors is shown below.

	P_{2X}	P_{2Y}	P_{2Z}
Type	Ligand-gated channel	G-protein coupled	Non-selective pore
Subtype	P_{2X1}, P_{2X2}, P_{2X3}	P_{2Y1}, P_{2Y2}, P_{2Y3}	

Various P_2 receptors have previously been cloned. P_{2Y1} was cloned by the Barnard/Burnstock group (Webb *et al.*, *FEBS Lett.* 324 219-225 (1993)) based on homology with

other 7-TM G-protein coupled receptors. This group used PCR technology and primers based on conserved domains of the second and sixth transmembrane regions to screen a mammalian brain cDNA library and, with final success, an embryonic chick whole-brain cDNA library.

P_{2Y2}/P_{2U} was cloned by the Julius laboratory (Lustig *et al., Proc. Nat'l. Acad. Sci. USA* 90 5113-5117 (1993)) by expression cloning in the oocyte from cDNA obtained from a NG108-15 neuroblastoma cell line.

P_{2Y3}/P_{2T} was also obtained by the Barnard/Burnstock group using the same probes and embryonic brain cDNA library used to obtain the P_{2Y1} receptor (Barnard *et al., Trends Pharmacol. Sci.* 15 67-70 (1994)).

However, as yet, cloning of the P_{2X} receptor has remained an elusive goal. The prior cloning exercises undertaken for the other P_2 receptors do not provide an adequate lead to enable the P_{2X} receptor to be cloned. First, all the above purinoceptors are G-protein coupled 7-TM proteins. Their myriad functions (like those of all 7-TM receptors) occur through G-protein activation of one or more second messenger systems. There are over 200 currently identified proteins which belong to this 7-TM/G-protein coupled family. Agonists at these receptors activate cascades of intracellular transduction pathways, often involving several enzymes; the response of the cell is inherently slow (several seconds to minutes) and changes in excitability are subtle if they occur. In contrast, the P_{2X} receptor is a fundamentally different type of purinoceptor that incorporates an ion channel. Activation of P_{2X} receptors is rapid (milliseconds), has predominately local effects, and brings about immediate

depolarisation and excitation.

Secondly, the tissue distribution of the P_{2X} receptor is distinctly different from other purinoceptors, and the physiological roles differ from other purinoceptors.

One of the principal established ways to clone a receptor is based on sequence relatedness of the nucleotides that encode the amino acids of the receptor protein; it depends on there being a fairly high level of homology between a known sequence and that of the unknown receptor. This method was used to clone the P_{2Y1} form (above). Several laboratories, including that of the applicants, invested significant effort in obtaining the P_{2X} receptor using PCR techniques and primers based on conserved regions of various ligand-gated ion channels (ie. nicotinic ACh, GABA, glutamate, 5-HT_3). This approach failed. With hindsight, this failure can be rationalised, as it can now, but only now, be seen that the structure of the P_{2X} receptor bears no homology with any of these ligand-gated ion channels. For the same reason, approaches based on fragment hybridisation would not succeed.

However, by adopting a different approach, it has now been found possible to clone the P_{2X} receptor, and it is on this achievement that the present invention is in part based.

According to a principal aspect of the present invention, there is provided a recombinant or isolated DNA molecule encoding a P_{2X} receptor, wherein the receptor:

(a) has the amino sequence shown in Figure 1, Figure 2, Figure 3 or Figure 4; or

(b) is substantially homologous to the sequence shown in Figure 1, Figure 2, Figure 3 or Figure 4;

or a fragment of such a DNA molecule, which fragment includes at least 15 nucleotides taken from nucleotides 1 to 813 shown in Figure 1, the full nucleotide sequences shown in Figures 2 and 3, or from nucleotides 1 to 1744 shown in Figure 4.

The sequence shown in Figure 1 is a cDNA sequence that encodes a rat vas deferens P_{2X} receptor. This sequence is 1837 bases in length and encodes a protein of 399 amino acids. As was determined after the receptor was cloned, approximately one half of the protein-encoding sequence, from nucleotides 814 onwards, had been discovered previously but the function of the previously cloned sequence was not known except that it appeared to be implicated in apoptotic cell death (Owens *et al.*, *Mol. Cell. Biol.* 11 4177-4188 (1991)); the Owens *et al.* sequence lacks a translation initiation site and could not be made into protein. (In Figure 1, the upstream portion of the reported sequence of Owens *et al.*, namely PQLAHGCYPCPPHR, which is not shared with the P_{2X} receptor, is shown for comparative purposes and does not form part of the invention.)

Preferably the Figure 1 sequence fragments are taken from nucleotides 1-810. Often the Figure 4 sequence fragments are taken from nucleotides 1-777.

The sequence shown in Figure 2 is a cDNA sequence that encodes a rat superior cervical ganglion P_{2X} receptor.

The sequence shown in Figure 3 is a cDNA sequence that encodes a rat dorsal root ganglion P_{2X} receptor.

The sequence shown in Figure 4 is the cDNA sequence that encodes a human P_{2X} receptor. The cDNA was isolated from the human urinary bladder using a rat P_{2X} probe. It is 2643 bases long and encodes a 399 amino acid protein having an amino acid sequence which is highly homologous with the amino acid sequence of the rat P_{2X} receptor isolated from rat vas deferens and with the rat P_{2X} receptors isolated from a rat superior cervical ganglion and from a rat dorsal root ganglion. Recently we have become aware of an expressed sequence tag corresponding to residues 1745-1933 (Proc. Natl. Acad.Sci. USA 91,10645-10649 (Oct. 1994).

Sequences which are substantially homologous to the Figure 1, Figure 2, Figure 3 or Figure 4 amino acid sequence include those which encode proteins having at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% homology in increasing order of preference. A protein having at least 99% homology with the amino acid sequence of Figure 1, Figure 2, Figure 3 or Figure 4 will have no more than four amino acid variations from such a sequence. Preferred substantially homologous sequences include P_{2X} sequences from other species. Thus for the rat P_{2X} receptor sequences a preferred substantially homologous sequence is a human P_{2X} sequence. One method of determining sequence homology is disclosed in WR Pearson and DJ Lipman, *Proc Natl Acad Sci USA* 85:2444-2448 (1988).

Fragments may of course be larger than 15 nucleotides. Fragments encoding substantially the whole of the P_{2X} rat receptors or human receptor may be expected to share the biological activity of the receptor, or at least some of its biological activities. Shorter fragments may be useful for encoding one or more selected domains of the receptor, or simply as probes for detecting or identifying other useful DNA sequences, including those encoding substantially homologous proteins. Fragments of

at least 20, 30 or 50 nucleotides may be more frequently of use than shorter ones.

DNA molecules of the invention are useful for a number of purposes. First, and not least, the P_{2X} cDNA shown in Figure 1, in Figure 2, in Figure 3 and in Figure 4 enables the relevant proteins to be expressed in living cells. This would not be possible with fragments of the cDNA. However not only are fragments of DNA within the scope of the invention, for the various purposes mentioned above, but also genomic and other sequences of DNA (including synthetic DNA and "minigenes", which include at least one, but not all, of the introns naturally present in the gene) are included within its scope. cDNA sequences encoding the rat receptor proteins or human P_{2X} receptor protein may be preferred in some circumstances because such sequences are smaller than either genomic or minigene DNA and therefore more amenable to cloning manipulations. The P_{2X} receptor protein can be stably expressible in chinese hamster ovary (CHO) cells, as will be described below.

Still on the subject of expression, while it would be possible to express genomic DNA in eukaryotic cells, it is much more difficult to manipulate the DNA for insertion into host cells due to the larger size that commonly results from introns. The size is particularly important for the expression of RNA; very long cRNAs -- the size of whole genes -- are difficult to make in sufficient quantity. On the other hand, expression from RNA is much preferred at least for the investigation of ion channel proteins, because the *Xenopus* oocyte is sufficiently large to be studied easily by electrophysiological methods.

Secondly, the cDNA sequences encode proteins that, in their predicted folding within the membrane, differ from other known proteins. This is advantageous because, based on historical precedent, this will lead to the discovery of a large family of related proteins and these may have functional roles unrelated to signalling mediated by ATP.

Thirdly, knowledge of the protein sequences encoded by rat and human P_{2X} cDNA allows the development of molecular models that predict the detailed disposition within the membrane. It further allows the correctness of such models to be determined by expression of mutagenised proteins. These two approaches are advantageous because they may permit the molecular design of complementary therapeutic agents that activate or block the receptor.

Fourthly, the P_{2X} cDNA sequences allow the distribution of the RNA that encodes this receptor, as well as the receptor protein itself, to be mapped in human tissues. RNA distribution can be determined by *in situ* hybridisation. Such hybridisation studies are disclosed in the present examples. Knowledge of a deduced amino acid sequence from cDNA allows synthetic peptides to be made that can be used to generate antibodies that selectively recognise a P_{2X} receptor. Thus a P_{2X} protein can be mapped by immunohistochemistry. This may suggest novel therapeutic applications for drugs that activate or block the P_{2X} receptor, that can not be predicted on the basis of less sensitive current methods for localising the receptor (radioactive ligand binding).

Fifthly, rat P_{2X} cDNA is advantageous because it can allow the isolation of a closely related cDNA from human tissue.

Sixthly, the isolation of the human P_{2X} cDNA clone will enable a human genomic clone to be obtained. It is probable that mutations of this gene will be discovered that lead to human genetic disease. The analysis of such mutations may lead to appropriate treatments of diseases or disorders caused by such mutations.

In one aspect of the present invention rat vas deferens P_{2X} receptor was cloned by a method which does not require prior inference about structure. Tissues were chosen that were believed to be rich in the RNA for the receptor of interest. A number of tissue sources were tried but they did not provide RNA that led to ATP responses in oocytes. Eventually, vas deferens was chosen. From extracted polyadenylated RNA, a cDNA library or bank that corresponds as far as possible to the DNAs in the tissue was constructed. It was not assured, either before work began or until it was satisfactorily completed, that a satisfactory cDNA library in which the rat P_{2X} gene was represented could be constructed; nevertheless, this was achieved in plasmid pBKCMV.

An individual clone within the library that contains the rat vas deferens P_{2X} cDNA of interest was detected by progressive fractionation of the library; at each step the fraction was tested to determine whether RNA made from it can direct the formation of the protein of interest. More specifically, RNA was transcribed *in vitro* from the cDNAs in the library (approximately 2 million) and the RNA ("cRNA") mixture was injected into immature *Xenopus* oocytes. cRNA is very susceptible to inadvertent enzymatic degradation, so all procedures were carried out under sterile conditions. The cDNA pools were made by the miniprep procedure and therefore

contained large amounts of *E. coli* RNA; this difficulty was overcome by precipitating any RNA before the cRNA was transcribed.

Detection of the protein can in principle be done by radioactive ligand binding or by a functional response. The activation of G proteins in the *Xenopus* oocyte and the subsequent cellular response was used to obtain the P_{2Y2}/P_{2U} receptor. In the present work, a decision was made to use the opening of the integral ion channel of the P_{2X} as the response. Individual oocytes were screened two days after injection to determine whether they had made P_{2X} receptor protein in their membrane. This was done by recording the current flowing across the oocyte membrane when ATP (30 μM) was applied to the outside of the oocyte; if the P_{2X} receptor has been produced, a small transient current would be expected. However, testing for expression of the receptor was not straightforward, as some batches of oocytes exhibit responses to ATP because they naturally express other kinds of ATP receptor. This difficulty was overcome as follows: when an oocyte responded to ATP with the expected current this was further tested by blockade with a P_{2X} receptor antagonist (suramin). The cDNA fraction that gave led to the positive response in such an oocyte was further divided, and each fraction was again tested. Such progressive fractionation led to isolation of a single clone. The insert in the plasmid was sequenced; the sequence is shown in Figure 1. This sequence was used to design PCR primers which were used in the cloning of cDNA encoding a P_{2X} receptor from a rat superior cervical ganglion (see Figure 2). A similar procedure was then used in the cloning of cDNA encoding a P_{2X} receptor from a rat dorsal root ganglion (see Figure 3).

DNA in accordance with the invention will usually be in recombinant or isolated form and may be in the form of a vector, such as a plasmid, phagemid, cosmid or virus, and in some embodiments contains elements to direct expression of the protein, for example in a heterologous host. Non-expressible vectors are useful as cloning vectors.

Although DNA in accordance with the invention may be prepared synthetically, it is preferred that it be prepared by recombinant DNA technology. Ultimately, both techniques depend on the linkage of successive nucleotides and/or the ligation of oligo- and/or poly-nucleotides.

The invention enables, for the first time, P_{2X} receptor to be prepared by recombinant DNA technology and hence free from protein with which it is naturally associated or contaminated (such as the P_{2U} or, particularly, P_{2Y} receptor, or other ATP receptors or binding proteins), and this in itself forms another aspect of the invention. The protein will generally be associated with a lipid bilayer, such as a cell, organelle or artificial membrane. P_{2X} receptor prepared by expression of DNA in accordance with the first aspect may be glycosylated, but does not have to be. Generally speaking, receptor proteins and ion channels that are glycosylated will also function after carbohydrate removal or when expressed in cells that do not glycosylate the protein. However, there are often important quantitative differences in the function between the glycosylated and non-glycosylated protein. In the case of the rat vas deferens P_{2X} receptor, we believe that the native protein is glycosylated because it has a molecular weight of 62 kd

when purified from the rat vas deferens, as compared to the molecular weight of 45 kd for the cloned protein. Similar results were obtained for the human P_{2X} receptor (see later).

There are also several asparagine residues in the extracellular domain that are likely sites of sugar attachment.

Knowledge of the amino acid sequence of a P_{2X} receptor enables the protein or peptide fragments of it to be prepared by chemical synthesis, if required. However, preparation by expression from DNA, or at least translation from RNA, will usually be preferred.

Particularly useful peptide fragments within the scope of the invention include epitopes (which may contain at least 5, 6, 7, 10, 15 or 20 amino acid residues) of the P_{2X} receptor which are immunologically non-cross reactive with the RP-2 polypeptide disclosed in Owens *et al., loc. cit.*

A P_{2X} receptor, and fragments of it, can be used to prepare specific polyclonal and monoclonal antibodies, which themselves form part of the invention. Polyclonal and monoclonal antibodies may be prepared by methods well established in the art. Hybridoma and other cells expressing monoclonal antibodies are also within the invention.

RNA encoding a P_{2X} receptor, transcribable from DNA in accordance with the invention and substantially free form other RNAs, also forms part of the invention, and may be useful for a number of purposes including hybridisation

studies, *in vitro* translation and translation in appropriate *in vivo* systems such as *Xenopus* oocytes.

The invention also relates to host cells transformed or transfected with a vector as described above. Host cells may be prokaryotic or eukaryotic and include mammalian cells (such as COS, CHO cells and human embryonic kidney cells (HEK 293 cells)), insect cells, yeasts (such as *Saccharomyces cerevisiae*) and bacteria (such as *Escherichia coli*). Host cells may only give transient expression of the receptor, as in the case of COS cells, but for preference the host cells are stably transfected with the vector. Host cells which appropriately glycosylate the receptor are preferred. A CHO cell line or any other cell line that stably expresses a P_{2X} receptor can be used for electrophysiological, calcium-influx, calcium-imaging and ligand-binding studies. Host cells which do not express the receptor may still be useful as cloning hosts.

A P_{2X} receptor prepared by recombinant DNA technology in accordance with the invention has a number of uses, either *in situ* in a membrane of the expression host or in *in vitro* systems. In particular, the receptor can be used as a screen for compounds useful in a variety of human (or other animal) diseases and conditions, as will now be briefly described. Such compounds include those present in combinatorial libraries, and extracts containing unknown compounds (e.g. plant extracts).

Epilepsy. Epilepsy results from overexcitation of distinct neurones in specific regions of the brain, in particular in the hippocampus. Functional ATP P_{2X} receptors are known to be present in some hippocampal

neurones. If the P_{2X} receptors are expressed on inhibitory interneurons, then receptor agonists would be therapeutically useful. If the receptor is expressed on principal (pyramidal or granule) cells, then receptor antagonists will be useful. If will now be possible to determine which classes of neuron express the receptor.

Cognition Hippocampal neurones respond to ATP by activation of a P_{2X} receptor; these areas are of primary importance to cognition. It is now possible to determine the cellular localisation of the P_{2X} receptor with in the hippocampus; depending on this localisation, either agonists or antagonists might be effective to enhance memory.

Emesis The acute trigger for emesis is rapid contraction of smooth muscle of the upper gastrointestinal tract. Activation of ATP P_{2X} receptors present on smooth muscle of the GI tract, in particular the stomach and trachea, results in strong, rapid muscle contractions. P_{2X}-antagonists selective for visceral smooth muscle could be useful for emesis. Furthermore, P_{2X} receptors are known to be expressed in the nucleus of the tractus solitarius (Ueno *et al.*, *J. Neurophysiol.* 68 778-785 (1992)) and may be involved in transmission from primary visceral afferents; this could be blocked by selective P_{2X} antagonists.

Pain First, P_{2X} receptors are expressed in dorsal horn neurones of the spinal cord. Activation of these neurones by ATP causes fast depolarizing, excitatory responses (Jahr & Jessell, *Nature* 304 730-733 (1983)); if a component of the transmission from nociceptive fibres is mediated by ATP then this could be blocked by

a P_{2X} antagonist. Secondly, ATP is one of the most noxious substance known when applied intradermally. This is because it activates directly the peripheral terminals of small diameter nociceptive fibres; it is known that the cell bodies in the dorsal root ganglion express P_{2X} receptors. A P_{2X} antagonist would be a peripherally active analgesic, and is likely to be effective in migraine.

Asthma Bronchial smooth muscles contract in response to activation of P_{2X} receptors. This may occur in response to ATP released from sympathetic nerves, or from local immune cells. P_{2X} antagonists may help to prevent stimulus-evoked spasms of bronchial smooth muscle and thereby reduce the frequency and/or severity of asthmatic attacks.

Peripheral vascular disease It is becoming clear that ATP and not noradrenaline is the primary vasoconstrictor neurotransmitter in small resistance arteries - those that comprise over 70% of total peripheral resistance. This has been shown for many vessels (Westfall *et al.*, *Ann. N.Y. Acad. Sci.* 603 300-310 (1991)). A selective antagonist could be used for local collateral vasodilation.

Hypertension Hypertension that is associated with increased sympathetic tone could be treated with P_{2X} receptor antagonists, because ATP is a major excitatory transmitter to many resistance vessels in several species including man (Westfall *et al.*, *loc. cit.* and Martin *et al.*, *Br. J. Pharmacol.* 102 645-650 (1991)).

Diseases of the immune system A molecule identical to part of the P_{2X} receptor has been cloned from thymocytes that have been induced to die (Owens *et al.*, *loc. cit.*).

The selective expression in these conditions implies that a molecule closely related to the P_{2X} receptor plays a role in the apoptosis that is an integral part of the selection of immunocompetent cells. The molecule described by Owens *et al.* (RP-2) was incomplete and could not have been translated into protein. The cloning of the P_{2X} receptor will now allow the isolation of full length RP-2 clones, their heterologous expression and the determination of their functional roles.

Irritable bowel syndrome ATP is an important transmitter to the smooth muscles of the intestinal tract, particularly in the colon. It is also a transmitter between neurons in the enteric nervous system, by activating P_{2X} receptors (Galligan, *Gastroenterology*, in press). Antagonists at P_{2X} receptors may therefore have utility in the management of this condition.

Premature ejaculation This could be prevented by preventing stimulus-evoked contraction of vas deferens smooth muscle. P_{2X} receptors are highly expressed in this tissue; antagonists at this site would prevent vas deferens contractility during sympathetic excitation.

Cystitis P_{2X} receptors may be implicated in increased bladder sensitivity in patients with cystitis. Thus antagonists of such P_{2X} receptors may be useful in treating cystitis.

Useful agonists and antagonists identified as described above also form an aspect of the invention.

The cloning of the hP_{2X} receptor is an important aspect of the present invention. hP_{2X} is the first human member of

a multigene family of ionotropic purinoceptors. Its strong similarity with P_{2X}, isolated from rat vas deferens and with P_{2X} isolated from rat superior cervical ganglion or from rat dorsal root ganglion, suggests that it is a human homolog of the rat proteins. The present inventors have found that differences between these two sequences are nearly all conservative substitutions of hydrophilic residues. Surprisingly, hP_{2X} has only 41% identity with the other reported P_{2X} receptor, that from rat PC12 cells (Brake *et al*, New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor *Nature* 371: 519-523 (1994)). The PC12 derived receptor was proposed to have a similar membrane topography and shares the conserved spacing of cysteine residues, indicated for the two smooth muscle sequences in Figure 5.

The computed molecular weight of the hP_{2X} polypeptide (45 kd) agrees with that of the *in vitro* translation product when made in absence of pancreatic microsomal membranes. A larger product, 60 kd, produced in presence of microsomes suggests glycosylation and supports the idea of a central extracellular domain. The predicted hP_{2X} protein thus has the general features of other cloned members of this family (Valera *et al*, A new class of ligand-gated ion channel defined by P_{2X} receptor for extracellular ATP *Nature* 371: 516-519 (1994); Brake - *supra*): a large, cysteine-rich extracellular central domain flanked by two transmembrane spans and short internal N- and C-termini.

The distribution of the hP_{2X} mRNA was examined by northern blot analysis. Hybridisation of a principal 2.6 kb species was seen in all RNA samples tested, with the exception of brain. A smaller, 1.8 kb band, observed in

spleen, and lung mRNAs could be due to a shorter 3' untranslated portion of the mRNA, as occurs for P_{2X} mRNA from the rat vas deferens. The hybridisation observed in thymus, lung, spleen and liver RNA may reflect the content of smooth muscle in those organs. However, hP_{2X} is likely to have roles in other cell types, as demonstrated by its presence in adrenal gland, and the hemopoetic cell line HL60. The strong induction of hP_{2X} mRNA by HL60 differentiation may reflect a parallel observation in rat in which the smooth muscle form of P_{2X} mRNA can be induced in immature thymocytes by dexamethasone (RP2 mRNA; Owens *et al*, Identification of mRNAs associated with programmed cell death in immature thymocytes *J J Molec Cell Biol* **11**: 4177-4188 (1991)).

The present invention has enabled the first comprehensive pharmacological characterization of a cloned P_{2X}-purinoceptor to be made. The time course of the responses to ATP and the sensitivity to α,β,-methylene ATP are similar to those reported for the native hP_{2X} in urinary bladder (Inoue & Brading, Human, pig and guinea-pig bladder smooth muscle cells generate similar inward currents in response to purinoceptor activation *Br J Pharmacol*, **103**: 1840-1841 (1991)). Thus the functional properties of some native P_{2X} purinoceptors can be obtained by the expression of a single molecular species. The agonist induced current recorded from ooctyes expressing the hP_{2X} clone gives a direct measure of the activation of P_{2X}-purinoceptors in a system with low levels of endogenous ectonucleotidase activity. The agonist profile 2MeSATP$\geq$ATP>α,β,-meATP for hP_{2X} is similar to that of the cloned rat vas deferens P_{2X}-purinoceptor. The high potency of α,β,-meATP in whole tissue studies (α,β,-meATP >> 2MeSATP$\geq$ATP) probably reflects, its

resistance to ectonucleotidases.

The concentration-effect curves for ATP, 2MeSATP and 2-chloro-ATP were superimposable, indicating that these particular substitutions at the 2' position on the adenine ring do not affect agonist binding to the P_{2X}-purinoceptor. The agonist activity of AP_5A is likely to be because diadenosine phosphates (AP_5A, and AP_6A) released from the platelets can act as vasoactive agents through activation of P_{2X}-purinoceptors.

Preferred features of each aspect of the invention are as for each other aspect, *mutatis mutandis*.

The invention will now be illustrated by the following examples. The examples refer to the accompanying drawings, in which:

FIGURE 1 shows DNA and amino acid sequences of the rat vas deferens P_{2X} receptor as determined in Example 2. (SEQ ID NO 4).

FIGURE 2 shows DNA and amino acid sequences of a rat superior cervical ganglion P_{2X} receptor, as determined in Example 11. (SEQ ID NO 5).

FIGURE 3 shows DNA and amino acid sequences of a rat dorsal root ganglion P_{2X} receptor, as determined in Example 12. (SEQ ID NO 6).

FIGURE 4 shows DNA and amino acid sequences of a

human P_{2X} receptor as determined in Example 6. (SEQ ID NO 7)

FIGURE 5 shows the alignment of the predicted amino acid sequence of hP_{2X} with the rat vas deferens P_{2X}, and *in vitro* translation of hP_{2X} protein.

TM1 and TM2 filled boxes indicate the hydrophobic regions and boxed amino acids indicate the differences between the two sequences,

o indicates conserved cysteine residues.
* Indicates potential sites of N-glycosylation.

FIGURE 6 shows an SDS-PAGE analysis of ^{35}S-methionine labelled hP_{2X} protein. Lanes 1 and 2 show *in vitro* coupled transcription/translation of pBKCMV-hP_{2X} cDNA in the absence and presence of microsomal membranes, respectively.

FIGURES 7 AND 8 show Northern analyses of the hP_{2X} cDNA, wherein:

A) FIGURE 7 shows Northern blot with 8 µg of total RNA from differentiated HL60 cells.

0 indicates HL60 cells without treatment;
PMA2 and PMA3 indicate respectively cells treated 2 days, and 3 days with PMA;
DMSO indicates cells treated 6 days with DMSO;
dcAMP indicates cells treated 5 days with dibutryl

cAMP;
UB indicates 100 ng of polyA$^+$ RNA from human urinary bladder; and

B) FIGURE 8 shows distribution of hP_{2X} in human tissues. Lanes contained 1 μg polyA$^+$ RNA except for the urinary bladder which contained 0.2 μg of polyA$^+$ RNA.

FIGURES 9, 10 and 11 show the response of oocytes expressing hP_{2X} to purinoceptor agonists, wherein:

A) FIGURE 9 shows traces which show inward currents evoked by ATP, 2 me SATP and α,β, me ATP (0.1, 1, and 100 μM). Records for each agonist are from separate oocytes;

B) FIGURE 10 shows concentration response relationships of full P_{2X}-purinoceptor agonists. Data are expressed relative to the peak response to 100 μM ATP; and

C) FIGURE 11 shows concentration response of partial P_{2X}-purinoceptor agonists. Data are fitted with a Hill slope of 1 (n = 4-8).

FIGURES 12 and 13 show the effects of P2-purinoceptor antagonists of hP_{2X} mediated responses, wherein:

A) FIGURE 12 shows concentration response curves for ATP in the presence of the P2-purinoceptor

agonist suramin (1, 10 and 100 μM) (n = 4 for each point); and

B) FIGURE 13 shows concentration dependence of suramin, DIDS, PPADS and P5P in inhibiting the response to 10 μM ATP (n = 4 for each point).

FIGURE 14 shows the results of the functional characterisation of rat superior ganglion P_{2X} receptors (as encoded by clone 3, described in Example 10). These experiments provided electrical recordings from transfected HEK293 cells.

Top left: Superimposed currents evoked by ATP (30 μM) during the time are indicated by the bar. Holding potential was changed from -70 to 20 mV.

Top right: Peak current as a function of membrane potential.

Bottom left: Superimposed currents evoked by ATP, from 1 to 300 μM.

Bottom right: Concentration-response curves for ATP and αβmethylene-ATP (points are mean ± s.e. mean for 5 - 8 experiments).

FIGURE 15 shows the inhibition of currents caused by various substances acting on the clone 3 form of the P_{2X} receptor (as described in Example 11), compared with PC12 and human bladder forms in HEK293 cells.

Top: inhibition by suramin.

Middle: inhibition by PPADS.

Bottom: inhibition by pyridoxal 5-phosphate.

EXAMPLES

(i) RAT VAS DEFERENS P_{2X} RECEPTOR

EXAMPLE 1 Cloning of the Rat vas deferens P_{2X} Receptor

Total RNA was isolated by the guanidinium isothiocyanate method (Sambrook *et al.*, "Molecular Cloning: A Laboratory Manual" Cold Spring Harbor Laboratory Press, second edition (1989)) from vas deferens of 4 weeks old Sprague-Dawley male rats, and the poly A+ RNA was subsequently purified by oligo(dT)-cellulose. First strand cDNA primed with the sequence 5'-GAGAGAGAGAGCGGCCGCTTTTTTTTTTTTTT-3' (SEQ ID NO 1) was synthesised with SUPERSCRIPT™ (BRL, Gaithersburg, MD, USA). After conversion of the cDNA to double stranded (Gubler & Hoffman, *Gene* 25 263-269 (1983)) *Eco*RI linkers were ligated to the cDNA, and the product was digested with *Not*I. The *Eco*RI-*Not*I cDNA of 1.3 to 9 kb was isolated by gel electrophoresis, and a unidirectional library was constructed by ligation of the cDNA to pBKCMV (Stratagene, San Diego, CA, USA) digested with the same enzymes. The library was electroporated into *E. coli* DH10B cells and divided in 24 pools of 8×10^4 clones. The plasmid DNA from the pools was prepared by minialkaline lysis followed by LiCl precipitation (Sambrook *et al.*, *loc. cit*). *Not*I-linearised cDNA was transcribed *in vitro* with T3 RNA polymerase in the presence of the cap analogue m7GpppG (Sambrook *et al.*, *loc. cit*). The *in vitro* transcribed RNA (cRNA) was concentrated to 4 mg/ml.

EXAMPLE 2 Sequencing of the Rat vas deferens P_{2x} Receptor cDNA

The cDNA insert was sequenced the exonuclease method (Henikoff *Meth. Enzymol.* 155 156-164 (1987)). The sequence is shown in Figure 1.

EXAMPLE 3 Functional characterisation of the Rat vas deferens P_{2x} Receptor cDNA in Oocytes

50 nl (200 ng) of RNA was injected into defolliculated *Xenopus* oocytes. After incubation for 2-6 days at 18°C, the oocytes were assayed for ATP-evoked currents by a two-electrode voltage clamp (GeneClamp™); one electrode is to hold the voltage constant (at -100 mV), and the other is to measure the currents. A cDNA pool which showed ATP induced currents was subdivided to obtain a single clone (P_{2X}). Electrophysiological measurements were done at -100 mV, in a perfusion medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes pH 7.6, and 5 mM sodium pyruvate. For dose-response curves and suramin inhibition, oocytes were injected with 100 ng P_{2x} cRNA, and all recordings were performed at -60 mV, with Ba^{2+} substituted for external Ca^{2+} to prevent activation of endogenous Ca^{2+}-activated Cl^- currents. Microelectrodes (0.5-2 MΩ) were filled with 3M KCl.

EXAMPLE 4 Functional characterisation of the Rat vas deferens P_{2x} Receptor cDNA in HEK 293 Cells

HEK 293 cells were transfected by the lipofectin method (Felgner *et al., Proc. Nat'l. Acad. Sci. USA* 84 7413-7417 (1987)) with P_{2x}-plasmid. DNA concentration used was 1 mg/2 ml medium placed into a 35 mm petri dish containing four 11 mm diameter coverslips on which HEK cells were placed at 10,000 cells per coverslip. Cells were exposed to lipofectin/DNA for 6 h and recordings made 16 - 36 h

later; 40 - 60% of cells from which recordings were made exhibited P_{2X} responses. Currents were recorded from HEK 293 cells using whole-cell recording methods and the AXOPATCH™ 200 amplifier (Axon Instruments); patch pipettes (5 MΩ) contained (mM) Cs or K aspartate 140, NaCl 5, EGTA 11, HEPES 5. The external solution was (mM) NaCl 150, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5 and glucose 11; the pH and osmolarity of both solutions were maintained at 7.3 and 305 mosmol/l respectively. All recordings performed at room temperature. Data acquisition and analysis were performed using pCLAMP™ and AXOGRAPH™ software (Axon Instruments). Solutions for experiments examining calcium permeability of ATP currents in HEK cells contained (mM): internal solution NaCl 150, HEPES 5, $CaCl_2$ 0.5 and EGTA 5 (free calcium concentration about 5 nM); external sodium solution NaCl 150, glucose 11, histidine 5, $CaCl_2$ 2; external calcium solution $CaCl_2$ 115, glucose 11 and histidine 5. The pH and osmolarity of the solutions were 7.4 and 295 mosmol/l respectively. For single channel measurements, a GENECLAMP™ 500 amplifier and outside-out recording methods were used (Adelman *et al.*, *Neuron* 9 209-216 (1992)). Wax-coated patch pipettes (5 - 10 MΩ) contained (mM) K-gluconate 115, HEPES 5, BAPTA 5 and $MgCl_2$ 0.5, external solution was 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes pH 7.6, and 5 mM sodium pyruvate. ATP was applied by U-tube typically for 1 s; data was sampled at 5 kHz in 2 s segments beginning 300 ms prior to onset of agonist (ATP) application and filtered at 1 kHz.

EXAMPLE 5 Transfection of the Rat vas deferens P_{2X} Receptor cDNA into CHO and HEK293 Cells

CHO cells were stably transfected by a method used for other ion channels (Claudio, *Meth. Enzymol.* 207 391-408

(1992)). Transfection was confirmed by a) electrophysiological recording and b) radioligand binding. ATP and other agonists (up to 30 μM) caused rapidly desensitising inward currents in 14 of 14 CHO cells stably transfected, and had no effect in 45 of 45 non-transfected cells. [^{3}H]αβmethyleneATP binding was more than 600 cpm per million transfected cells with less than 80 cpm nonspecific binding.

Stable transfection of HEK293 cells was also achieved. This was confirmed by electrophysiological recording.

(ii) HUMAN P_{2X} RECEPTOR

The materials and methods used in the human P_{2X} receptor examples are set out below:

In Vitro translation *In vitro* coupled transcription/ translation were performed using Promega's TNT Coupled reticulocyte lysate Systems with or without 2 µl of canine pancreatic microsomal membranes (Promega). µg Circular pBKCMV-hP_{2X} (0.5 ug) was transcribed with the T3 RNA polymerase as described in the system manual in a 25 µl reaction for 2 h are 30°C. Synthesized proteins (5 µl) were analysed by SDS-PAGE and autoradiography.

Differentiation of HL60 cells HL60 cells (human promyelocytes ATCC CCL240) were passaged twice weekly in RPMI-1640 supplemented with 25 mM HEPES, 2 mM Glutamax II, and 10% heat-inactivated fetal calf serum (GIBCO BRL). For each experiment 33 x 10^6 cells were resuspended at 2.5 x 10^5 cells/ml in medium containing either phorbol mystate acetate (100 nM), 1.1% DMSO, or dibutyryl cAMP (200 uM) (SIGMA) for the indicated times.

Northern blot analysis PolyA$^+$ RNAs were obtained from Clontech Laboratories Inc. (Palo Alto) except for the urinary bladder and HL60 mRNA which were prepared as described (Valera *et al* (1994) - *supra*). Samples were quantified by measuring the O.D. at 260 nm, and by staining the membrane with methylene blue. The RNA were fractionated on a 1% agarose - 6% formaldehyde gel and electroblotted to a non-charged nylon membrane (BDH). Prehybridisation at 68°C was performed for 6 hours in hybridisation buffer (50% formamide, 5X SSC, 2% blocking buffer (Boehringer Mannheim), 0.1% laurolylsarcosine,

0.02% SDS). Hybridisation was overnight at 68°C in fresh hybridisation buffer with a digoxigenin-UTP labelled riboprobe (100 ng/ml) corresponding to the entire hP_{2X} sequence. The membrane was washed at 68°C; twice in 2X SSC + 0.1% SDS, and twice in 0.1X SSC + 0.1% SDS. Chemiluminescent detection of hybridisation was carried at room temperature as follows: the membrane was rinsed 5 min in buffer B1 (0.1 M maleic acid, 0.15 M NaCl, pH 7.5), saturated for 1 hour in 1% blocking buffer (B2), incubated 30 min with anti-digoxigenin-antibody alkaline phosphatase conjugated (750 u/ml, Boehringer Mannheim) diluted 1:15000 in B2, washed in B1 + 0.3% tween 20 (1X 5 min, 1X 15 min, 1X 1 h), equilibrated for 5 min in buffer B3 (0.1 M Tris HCl pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$), incubated 45-60 sec in lumigen PPD (Boehringer Mannheim) diluted 1:100 in B3. The humid membrane was sealed in a plastic bag, incubated 15 min at 37°C, and exposed 15 to 20 min to Hyperfilm-ECL (Amersham).

P_{2X} expression into oocytes Human urinary bladder P_{2X} cDNA, subcloned into the pBKCMV expression vector, was linearized with NotI, and transcribed *in vitro* with T3 polymerase in the presence of cap analogue m7G(5')ppp(5')G. Defolliculated *Xenopus* oocytes (Bertrand *et al*, Electrophysiology of neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes following nuclear injection of genes or cDNAs *Meth Neurosci* 4: 174-193 (1991)) were injected with 50 ng of human P_{2X} *in vitro* transcribed RNA, and incubated at 18°C for 2-6 days in the ND96 solution (mM): NaCl96, KCl2, $MgCl_2$ 1, $CaCl_2$ 2, sodium pyruvate 5, HEPES 5, ph 7.6 - 7.5, penicillin (10 U/ml), and streptomycin (10 µg/ml).

Electrophysiology Oocytes were placed in a 1 ml chamber and superfused at 2 - 3 ml/min with ND96 solution with 0.1 mM $BaCl_2$ replacing the 2 mM $CaCl_2$ to prevent activation of endogenous calcium-activated chloride currents (Barish, A transient calcium-dependent chloride current in the immature *Xenopus* oocytes *J Physiol* 342: 309-325 (1983)). Currents were measured using a two-electrode voltage-clamp amplifier (Geneclamp Axon Instruments) at a holding potential of -60 mV. Microelectrodes were filled with 3 M KCl (0.5 - 2 MΩ). Data were collected using PClamp software (Axon Instruments). ATP and other purinoceptor agonists were applied by a U-tube perfusion system (Fenwick *et al*, A patch clamp study of bovine chromaffin cells and their sensitivity to acetylcholine *J Physiol* 331: 577-597 (1982)) placed close (200 - 500 μm) to the oocyte. Initial studies showed that reproducible responses (<10% variation in peak amplitude) could be obtained when ATP (at concentrations up to 1 mM) was applied to hP_{2X} injected oocytes for 5 s every 10 mins. Concentration response relationships to ATP and its analogs were determined by measuring the peak amplitude of responses to a 5 s application of agonist applied at 10 min intervals. Responses to agonists were normalized in each oocyte to the peak response evoked by 100 μM ATP; 100 μM ATP was usually applied at the beginning and at the end of an experiment to determine if there was any rundown of the response. No inward current was recorded in uninjected oocytes in response to application of purinoceptor agonists at the maximal concentration used (n = 3 for each agonist). Antagonists were applied both in the superfusate and together with ATP in the U-tube solution. Antagonists were superfused for 5 - 10 min prior to the application of ATP.

Data analysis Concentration response curves for purinoceptor agonists were fitted with a Hill slope of 1. Equi-effective concentrations i.e. concentration of agonist, giving 50% of the response to 100 μM ATP, (EEC_{50}) were determined from individual concentration response curves. For antagonists the concentration required to give 50% inhibition (IC50) of the response to 10 μM ATP (approximately 90% of peak response to ATP) were determined. Data are presented throughout as mean ± SEM for a given number of oocytes.

Drugs Adenosine, adenosine 5'-monophosphate sodium salt (AMP), adenosine 5'-diphosphate sodium salt (ADP), adenosine 5'-triphosphate magnesium salt (ATP), adenosine 5'-O-(-3-thiophosphate) tetralithium salt (ATP-γ-S), uridine 5'-triphosphate sodium salt (UTP), α,β-methylene ATP lithium salt (α,β,-meATP), β,γ-methylene-D-ATP sodium salt (D-β,γ-meATP), 2'-3'-O-(4-benzoylbenzol)ATP tetraethylamonium salt (BzATP), 4,4'-diisothiocyanatostilbene 2,2'-disulphonic acid, disodium salt (DIDS) were obtained from Sigma. 2-MethylthioATP tetra sodium salt (2MeSATP), 2-chloro-ATP tetra sodium salt, and β-γ-methylene-1-ATP (1-β-γ-meATP) were obtained from RBI. Pyridoxal 5-phosphate monohydrate (Aldrich), p1, p5-di[adenosine-5']pentaphosphate trilithium salt (AP5A) (Boehringer Mannheim), pyridoxal phosphate 6-azophenyl 2',4'-disulphonic acid (PPADS, gift of G. Lambrecht, University of Frankfurt) and suramin (Bayer) were tested. Drugs were prepared from frozen aliquots of stock solutions and diluted to give the required final concentration.

EXAMPLE 6 Sequence and characteristics of hP_{2X} from urinary bladder

Isolation of human P_{2X} cDNA Human urinary bladder tissue was obtained from a cystectomy for a bladder tumor. The patient showed no symptoms of bladder instability or urodynamic abnormalities. Only those portions, surrounding the tumor, which appeared macroscopically normal (Palea *et al* - *supra*) were used. Total RNA was isolated by guanidinium isothiocyanate and poly A^+ RNA was purified as described (Valera *et al* (1994) - *supra*). Preparation of a cDNA library in λgt10, random primer labelling of a rat smooth muscle P_{2X} probe (Valera *et al* (1994) - *supra*), low stringency hybridisation screening and lambda phage DNA isolation were all done by standard protocols (Sambrook *et al*, Molecular Cloning, A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, New York (1989)). Several independent phage isolates were examined and the cDNA insert from one was chosen for subcloning into Eco RI-Not I digested pBKCMV. This 2677 bp hP_{2X} cDNA was sequenced as described (Valera *et al* (1994) - *supra*).

The 2677 bp cDNA, hP_{2X}, contained a single long open reading frame which corresponds to a protein of 399 amino acids (Figure 4). This amino acid sequence is highly homologous with that of the P_{2X} receptor, isolated from rat vas deferens (89% identity). There are two regions of hydrophobicity near either end of the protein which are sufficiently long to traverse the membrane but there is no hydrophobic N-terminal leader sequence. All five potential sites for glycosylation and all ten cysteine residues in the central section of the protein are conserved. *In vitro* translation of hP_{2X} RNA in the

presence of microsomes produced a 60 kD product, whereas translation in the absence of microsomes produced the 45 kD peptide (Figure 6). 45 kD is the computed molecular weight, suggesting that the additional 15 kD results from glycosylation.

Some human urinary bladder P_{2X} cDNA was used to transfect HEK293 cells. Stable transfection was confirmed by electrophysiological recording.

EXAMPLE 7 Distribution of human urinary bladder P_{2X} mRNA

The distribution of the human urinary bladder P_{2X} mRNA was examined by northern analysis. A single 2.6 kb mRNA species was observed in bladder, placenta, liver and adrenal gland (Figure 8). In thymus, spleen, and lung samples, the 2.6 kb band plus additional higher molecular weight RNAs of 3.6 and 4.2 kb were seen. A smaller additional RNA species of 1.8 kb was observed in spleen and lung. No hybridisation was detected with brain mRNA.

EXAMPLE 8 Induction of hP_{2X} mRNA in HL60 cells

A portion of the 3'-untranslated region had been previously deposited in the database (HSGS01701) as an expressed sequence tag for the differentiation of the human promyelocytic cell line, HL60 (Okubo unpublished). We examined the induction of hP_{2X} mRNA in HL60 cells by Northern blot analysis (Figure 7). HL60 cells can be differentiated into distinct lineages, depending on the inductant (Koeffler, Induction of Differentiation of Human Acute Myelogenous Leukemia Cells: Therapeutic Implications *Blood* 62: 709-721 (1983)). Induction of macrophage-like characteristics with phorbol diesters or

granulocytic differentiation with DMSO or dibutryl cAMP, each produced an increase in P_{2X} mRNA (Figure 7, lane 6), HL60 RNA (lane 1-5) showed hybridisation of two bands (1.8 and 2.6 kb) and both of these were inducible. This contrasts with the bladder, where Northern analysis showed only a single RNA species (2.6 kb) (Figure 7, lane 6).

EXAMPLE 9 Pharmacological characterization of hP_{2X}

Application of ATP (30 nM - 1mM) to oocytes injected with hP_{2X} receptor RNA evoked inward currents (Figures 9, 10 and 11). Responses to low concentrations of ATP (30 - 300 nM) developed over 3-5 s. Higher concentrations of ATP (1 μM) evoked responses which peaked within 1 - 1.5 s and then declined during the continued application of ATP (40 - 60% of the peak amplitude after 5 s). The current returned to control values on washout of ATP. The peak amplitude of the inward current evoked by ATP was concentration-dependent (Figures 9, 10 and 11) and could be fitted by a curve with a Hill slope of 1 with a EC_{50} of 0.82 μM. When ATP (100 μM) was applied for 5 s every 10 min, reproducible inward currents were recorded. This is in contrast to the responses of the P_{2X} receptor clone from rat vas deferens where a second application of ATP (>1 μM) applied 10 mins after the first, evoked an inward current that was ~50% of the initial peak amplitude.

Concentration-response curves were constructed for a number of other P2 purinoceptor agonists (Figures 9, 10 and 11). 2meSATP, 2-chloro-ATP, α,β-meATP and ADP were full agonists. BzATP, AP_5A and ATP-γ-S produced maximal responses of about 65% of the maximal ATP response. The

maximal responses to d and 1-β,γ-meATP were not determined. Adenosine, AMP and UTP (100 μM) evoked small inward currents (2.3 ± 1.5, 6.08 ± 2, and 3.7 ± 1.8% of the response to 100 μM ATP respectively). The EEC_{50} values and relative potencies of purinoceptor analogs are summarised in Table 1 below.

Table 1

agonist	EEC50 (μM)	relative potency
ATP	0.82	1
2MeSATP	0.6 ± 0.1	1.36
2chloroATP	0.76 ± 0.1	1.08
AP5A	2 ± 0.2	0.41
α,β-meATP	3.6 ± 1.6	0.23
BzATP	4.2 ± 2.2	0.20
ATP-γ-S	10.6 ± 3.8	0.077
d,β,γ-meATP	24.1 ± 1.6	0.034
ADP	34.3 ± 16	0.024

EEC50: Equi-effective concentrations producing an inward current equivalent to 50% of the peak response to 100 μM ATP. EEC50 taken from individual fitted concentration response curves with a Hill slope of 1. EEC50 for ATP from mean data from all experiments. (n = 3-4).

EXAMPLE 10 Antagonist studies

The P2-purinoceptor antagonist suramin (1 - 100 μM) shifted the concentration-response curve for ATP to the right. At 1 μM suramin the shift was almost parallel. The dissociation equilibrium constant (K_B) estimated from $K_B = 1/(DR-1)$ where DR is the dose ratio was 130 nM. With higher concentrations of suramin the inhibition did not

appear to be competitive. Under the present experimental conditions this K_B estimate is higher than those reported previously for suramin (pA2 5.9, Trezise *et al*, *Br J Pharmacol* 112: 282-288 (1994)) pK_B 5.2, von Kugelgen *et al*, Interaction of adenine nucleotides, UTP and suramin in mouse vas deferens: suramin-sensitive and suramin-insensitive components in the contractile effect of ATP *Naunyn Schmiedeberg's Arch Pharmacol* 342: 198-205 (1990)). The antagonism by suramin was fully reversed after 10 mins wash and indicates that the non-competitive antagonism at high concentrations is not due to irreversible binding of the antagonist to the receptor.

The putative P_{2X} purinoceptor antagonists PPADS, DIDS and pyridoxal 5 phosphate (Ziganshin *et al*, Selective antagonism by PPADS at P_{2X} purinoceptors in rabbit isolated blood vessels *Br J Pharmacol* 111: 923-929 (1994), Bultmann & Starke, Blockade by 4,4'-diisothiocyanatostilben-2,2'-disulphonate (DIDS) of P_{2X} purinoceptors in rat vas deferens *Br J Pharmacol* 112: 690-694 (1994), Trezise *et al*, *Eur J Pharmacol* 259: 295-300 (1994)) inhibited inward currents evoked by 10 μM ATP (approximately EC_{90} concentration) in a concentration dependent manner (Figures 12 and 13). Suramin PPADS and DIDS were equally effective in inhibiting ATP evoked currents (IC_{50} ~ 1 μM). The IC 50 for P5P was ~ 20 μM. PPADS and P5P antagonism was readily reversible on washout. In contrast, inhibitory effects of DIDS (100 μM) were very slow to reverse on washout.

(iii) RAT SUPERIOR CERVICAL GANGLION P_{2X} RECEPTOR

Example 11 **Isolation and functional expression of a cDNA encoding a P_{2X} receptor from rat superior cervical ganglion (referred to herein as clone 3)**

A 440 bp fragment was amplified by polymerase chainreaction (PCR) from rat testis cDNA, using degenerate primers based on conserved nucleotide sequences within the rat vas deferens P_{2X} receptor cDNA and on the sequence of PC12 cDNA (Ehrlich H A (ed) *PCR Technology* MacMillan, Basingstoke (1989)). The primers used are given below:

Sense

5' T G T/C G A A/G A/G T I T T/C I G G/C I T G G T G T/C C C 3' (SEQ ID NO 2)

Antisense

5' G C A/G A A T/C C T A/G A A A/G T T A/G T/A A I C C 3' (SEQ ID NO 3)

(wherein I = Inosine and "T/C" indicates that either T or C is present at the position indicated (this applies *mutatis mutandis* to the other alternatives given).

The cloned PCR fragment was labelled and used as a hybridization probe for screening a rat testis cDNA bank in λZAP. One recombinant phage was positive, and its insert was excised and transferred to a plasmid (#432). This cDNA was 1500 bp with a single EcoR1 site (at position 1000, still in the open reading frame). The 5' end of the cDNA was too short to encode the entire N terminus.

Internal primers specific to the new sequence were made and the tissue distribution was tested by PCR. The candidate was present in mRNA prepared from phaeochromocytoma (PC12) cells, intestine and superior cervical ganglion (scg). The hybridization probe was therefore used to screen a rat scg cDNA bank in λgt10. From 30 initial positives, 20 pure phage DNA stocks were prepared; 19 were various portions of the candidate sequence, and the insert from one was transferred to plasmid (p457) and sequenced. The insert appeared to be a full length cDNA; it has a single open reading frame of 388 amino acids (Fig. 2). The insert from p457 was subcloned into pcDNA3 (p464) and used to transfect human embryonic kidney (HEK293) cells.

The functional characterisation of the clone illustrated in Fig 2 (referred to herein as clone 3) was carried out by electrical recordings from transfected HEK293 cells and from oocytes injected with the *in vitro* transcribed RNA, as described in Example 4 for the rat vas deferens P_{2X} receptor. Table A summarizes the main properties of clone 3 as compared to those of rat vas/human bladder cDNA clone, and the PC12 cDNA clone (provided by David Julius and Tony Brake of the University of California at San Francisco).

TABLE A

Functional Properties of 3 cloned P_{2x} Receptors

	bladder	clone 3	PC12
kinetics			
desensitization	very strong	very little	very little
rundown	profound	very little	very little
ionic permeability			
monovalent	no differences	no differences	no differences
divalent (Ca^{++})	high permeability	high permeability	high permeability
Ca^{++} block	none	intermediate	very strong
agonist profile			
ATP	0.7 μM	11 μM	8 μM
α,β-meATP	3 μM	>>100 mM	>>100 μM
antagonist profile			
suramin	1 μM	< 40% block	6 μM
PPADS	1 μM	< 30% block	1 μM
P-5-P	6 μM	< 40% block	6 μM
DIDS	1 μM		> 100 μM

The main functional properties of clone 3 are as follows. (a) The currents evoked by ATP show little or no decline during applications of several seconds; that is, there is little desensitisation (Fig. 14). (b) The relative permeabilities of the ionic pore to sodium, potassium, cesium, tetraethylammonium and to calcium are not different to those observed for the rat vas deferens/human bladder or the PC12 forms of the receptor. (c) Extracellular calcium (30 mM) inhibits the inward current through the P_{2X} receptor channel of the PC12 form whereas it does not block current through the rat vas deferens/human bladder form; clone 3 is intermediate in sensitivity. (d) The effectiveness of agonists that are structurally related to ATP is the same as that found for the PC12 form; most notably, $\alpha\beta$methylene ATP has little or no agonist action (Fig. 14). (e) Currents activated by ATP at the clone 3 receptor were much less sensitive to antagonism by suramin., pyridoxal 5'-phosphate and pyridoxal-6-azophenyl-2',4'-disulphonic acid (PPADS) than were similar current mediated by the other two forms (rat vas deferens/human bladder; PC12) (Fig. 15).

(iv) RAT DORSAL ROOT GANGLION P_{2X} RECEPTOR

Example 12 **Isolation of a cDNA encoding a P_{2X} receptor from a rat dorsal root ganglion**

By using PCR with the same primers as used in Example 11 above, but using different cDNA sources, further P_{2X} family members can be found.

Using this method, rat dorsal root ganglion P_{2X} receptor cDNA was isolated. Fig. 1B shows the cDNA sequence of this clone (referred to herein as clone 6), together with the putative amino acid sequence. The portions underlined in this figure correspond to the PCR primers initially used.

A similar procedure to that described in Example 11 was then used to isolate the full length cDNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(C) CITY: GREENFORD
(D) STATE: MIDDLESEX
(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): UB6 ONN

(ii) TITLE OF INVENTION: DNA AND PROTEIN SEQUENCES

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAGAGAGAGA GCGGCCGCTT TTTTTTTTT TTT 33

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:3
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "T or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:7
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:11
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "T or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:14
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "G or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:21
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "T or C"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TGNGANNTNT NNGNNTGGTG NCC

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:3
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "T or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:16
(D) OTHER INFORMATION:/mod_base= OTHER
/note= "T or A"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:18
(D) OTHER INFORMATION:/mod_base= i

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCNAANCTNA ANTTNNANCC 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1837 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: rat P2x from vas deferens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:210..1406

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
GCCAAAAGCT GTTCTGATCA CCCAGGGTTT TTCCTCCCAA CCCAGACCCC ACCATCGAAC      60

CTCCAACTCT GGTCCCACCT AGCCTGCTCT GTCCTTAAGG GGCCGGGAAG CCCCAGTCAC     120

TCCACTGCTA TTGTAGATGC AGATGGTGGC CTGCCCTTGA CCATAGAGGC CGTGTGGGGT     180

GTTCATCTCT GAGCCCCTTC TGGCCCACC ATG GCT CGG CGG CTG CAA GAT GAG        233
                                Met Ala Arg Arg Leu Gln Asp Glu
                                  1               5

CTG TCA GCC TTC TTC TTT GAA TAT GAC ACT CCC CGG ATG GTG CTG GTA        281
Leu Ser Ala Phe Phe Phe Glu Tyr Asp Thr Pro Arg Met Val Leu Val
     10                  15                  20

CGA AAC AAG AAG GTG GGA GTC ATT TTC CGT CTG ATC CAG TTG GTG GTT        329
Arg Asn Lys Lys Val Gly Val Ile Phe Arg Leu Ile Gln Leu Val Val
 25                  30                  35                  40

CTG GTC TAC GTC ATT GGG TGG GTG TTT GTC TAT GAA AAA GGA TAC CAG        377
Leu Val Tyr Val Ile Gly Trp Val Phe Val Tyr Glu Lys Gly Tyr Gln
                 45                  50                  55

ACC TCA AGT GAC CTC ATC AGC AGT GTG TCC GTG AAG CTC AAG GGC TTG        425
Thr Ser Ser Asp Leu Ile Ser Ser Val Ser Val Lys Leu Lys Gly Leu
             60                  65                  70

GCT GTG ACC CAG CTC CAG GGC CTG GGA CCC CAG GTC TGG GAC GTG GCT        473
Ala Val Thr Gln Leu Gln Gly Leu Gly Pro Gln Val Trp Asp Val Ala
         75                  80                  85

GAC TAT GTC TTC CCA GCA CAC GGG GAC AGC TCC TTT GTA GTT ATG ACC        521
Asp Tyr Val Phe Pro Ala His Gly Asp Ser Ser Phe Val Val Met Thr
     90                  95                 100

AAC TTC ATC GTG ACC CCT CAG CAG ACT CAA GGC CAT TGT GCA GAG AAC        569
Asn Phe Ile Val Thr Pro Gln Gln Thr Gln Gly His Cys Ala Glu Asn
105                 110                 115                 120
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CCA GAA GGT GGC ATA TGC CAG GAT GAC AGT GGC TGC ACT CCA GGA AAA      617
Pro Glu Gly Gly Ile Cys Gln Asp Asp Ser Gly Cys Thr Pro Gly Lys
                125                 130                 135

GCA GAA AGG AAA GCC CAA GGT ATT CGC ACA GGC AAC TGT GTG CCC TTC      665
Ala Glu Arg Lys Ala Gln Gly Ile Arg Thr Gly Asn Cys Val Pro Phe
            140                 145                 150

AAT GGC ACT GTG AAG ACA TGT GAG ATC TTT GGT TGG TGT CCT GTA GAG      713
Asn Gly Thr Val Lys Thr Cys Glu Ile Phe Gly Trp Cys Pro Val Glu
        155                 160                 165

GTG GAT GAC AAG ATC CCA AGC CCT GCT CTT CTT CGT GAG GCT GAG AAC      761
Val Asp Asp Lys Ile Pro Ser Pro Ala Leu Leu Arg Glu Ala Glu Asn
    170                 175                 180

TTC ACC CTC TTC ATC AAA AAC AGC ATC AGC TTT CCA CGC TTC AAG GTC      809
Phe Thr Leu Phe Ile Lys Asn Ser Ile Ser Phe Pro Arg Phe Lys Val
185                 190                 195                 200

AAC AGG CGC AAC CTG GTA GAG GAG GTG AAC GGC ACC TAC ATG AAG AAG      857
Asn Arg Arg Asn Leu Val Glu Glu Val Asn Gly Thr Tyr Met Lys Lys
                205                 210                 215

TGC CTC TAT CAC AAG ATT CAA CAC CCC CTG TGC CCA GTC TTC AAC CTT      905
Cys Leu Tyr His Lys Ile Gln His Pro Leu Cys Pro Val Phe Asn Leu
            220                 225                 230

GGC TAT GTG GTG CGA GAG TCA GGC CAG GAC TTC CGC AGC CTT GCT GAG      953
Gly Tyr Val Val Arg Glu Ser Gly Gln Asp Phe Arg Ser Leu Ala Glu
        235                 240                 245

AAG GGT GGG GTG GTT GGT ATC ACC ATT GAC TGG AAG TGT GAT CTG GAC     1001
Lys Gly Gly Val Val Gly Ile Thr Ile Asp Trp Lys Cys Asp Leu Asp
    250                 255                 260

TGG CAC GTT CGG CAC TGC AAA CCC ATC TAC CAG TTC CAC GGA CTG TAT     1049
Trp His Val Arg His Cys Lys Pro Ile Tyr Gln Phe His Gly Leu Tyr
265                 270                 275                 280

GGG GAG AAG AAC CTG TCT CCA GGC TTC AAC TTC AGA TTT GCC AGG CAT     1097
Gly Glu Lys Asn Leu Ser Pro Gly Phe Asn Phe Arg Phe Ala Arg His
                285                 290                 295

TTC GTG CAG AAT GGG ACA AAC CGT CGT CAC CTC TTC AAG GTG TTT GGG     1145
Phe Val Gln Asn Gly Thr Asn Arg Arg His Leu Phe Lys Val Phe Gly
            300                 305                 310

ATT CAC TTT GAT ATC CTT GTG GAT GGC AAG GCT GGG AAG TTT GAC ATC     1193
Ile His Phe Asp Ile Leu Val Asp Gly Lys Ala Gly Lys Phe Asp Ile
        315                 320                 325

ATC CCT ACT ATG ACT ACT ATC GGT TCT GGG ATT GGC ATC TTT GGA GTG     1241
Ile Pro Thr Met Thr Thr Ile Gly Ser Gly Ile Gly Ile Phe Gly Val
    330                 335                 340

GCC ACA GTG CTT TGT GAT CTC TTA TTG CTC CAC ATC CTG CCT AAG AGG     1289
Ala Thr Val Leu Cys Asp Leu Leu Leu Leu His Ile Leu Pro Lys Arg
345                 350                 355                 360
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CAC TAC TAC AAG CAG AAG AAG TTC AAA TAT GCC GAG GAC ATG GGG CCG      1337
His Tyr Tyr Lys Gln Lys Lys Phe Lys Tyr Ala Glu Asp Met Gly Pro
                365                 370                 375

GGA GAG GGT GAA CAT GAC CCC GTG GCC ACC AGC TCC ACT CTG GGC CTG      1385
Gly Glu Gly Glu His Asp Pro Val Ala Thr Ser Ser Thr Leu Gly Leu
            380                 385                 390

CAG GAG AAC ATG AGG ACC TCC TGACCTTAGT CTTGAGATCC GGACTTGACG         1436
Gln Glu Asn Met Arg Thr Ser
        395

CAGTGTGTGG CTTCCGGCAA GGGCTGATGG CTTTGAGCCA GGGCAGAGGG CATTCCCAGA    1496

GGCTTTCCTG CAAGGCAGAC ACCAGTGGCC CTCTGGTTCA GCATGAAGAC AGGCAAGACT    1556

TTGGATTTCA GAGCTCTGGT TTCAGTTCCA CATGTCCCTT CCTGAGGGAT GCCTCCTCCA    1616

GTTTTCACCA ATTTGGGTTC ATATGGCTGG GCCCCTCACA CATCTATACT CTAGCTTTGT    1676

GCTTAAGGCT CAGGCTGTCA TTGTCTTTCC CACAGCCTTA CCTGCCTAGA TTTGGGCTCT    1736

TCCACATGGT AGCCACTAGC CAGATGTGTC AGTTTGAACT TTAATTAAAA TATAATAAAA    1796

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A                        1837
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Arg Arg Leu Gln Asp Glu Leu Ser Ala Phe Phe Phe Glu Tyr
1 5 10 15

Asp Thr Pro Arg Met Val Leu Val Arg Asn Lys Lys Val Gly Val Ile
20 25 30

Phe Arg Leu Ile Gln Leu Val Val Leu Val Tyr Val Ile Gly Trp Val
35 40 45

Phe Val Tyr Glu Lys Gly Tyr Gln Thr Ser Ser Asp Leu Ile Ser Ser
50 55 60

Val Ser Val Lys Leu Lys Gly Leu Ala Val Thr Gln Leu Gln Gly Leu
65 70 75 80

Gly Pro Gln Val Trp Asp Val Ala Asp Tyr Val Phe Pro Ala His Gly
85 90 95

Asp Ser Ser Phe Val Val Met Thr Asn Phe Ile Val Thr Pro Gln Gln
100 105 110

Thr Gln Gly His Cys Ala Glu Asn Pro Glu Gly Gly Ile Cys Gln Asp
115 120 125

Asp Ser Gly Cys Thr Pro Gly Lys Ala Glu Arg Lys Ala Gln Gly Ile
130 135 140

Arg Thr Gly Asn Cys Val Pro Phe Asn Gly Thr Val Lys Thr Cys Glu
145 150 155 160

Ile Phe Gly Trp Cys Pro Val Glu Val Asp Asp Lys Ile Pro Ser Pro
165 170 175

Ala Leu Leu Arg Glu Ala Glu Asn Phe Thr Leu Phe Ile Lys Asn Ser
180 185 190

Ile Ser Phe Pro Arg Phe Lys Val Asn Arg Arg Asn Leu Val Glu Glu
195 200 205

Val Asn Gly Thr Tyr Met Lys Lys Cys Leu Tyr His Lys Ile Gln His
210 215 220

Pro Leu Cys Pro Val Phe Asn Leu Gly Tyr Val Val Arg Glu Ser Gly
225 230 235 240

Gln Asp Phe Arg Ser Leu Ala Glu Lys Gly Gly Val Val Gly Ile Thr
245 250 255

Ile Asp Trp Lys Cys Asp Leu Asp Trp His Val Arg His Cys Lys Pro
260 265 270

Ile Tyr Gln Phe His Gly Leu Tyr Gly Glu Lys Asn Leu Ser Pro Gly
275 280 285

Phe Asn Phe Arg Phe Ala Arg His Phe Val Gln Asn Gly Thr Asn Arg
290 295 300

Arg His Leu Phe Lys Val Phe Gly Ile His Phe Asp Ile Leu Val Asp
305 310 315 320

Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Thr Thr Ile Gly
325 330 335

Ser Gly Ile Gly Ile Phe Gly Val Ala Thr Val Leu Cys Asp Leu Leu
340 345 350

Leu Leu His Ile Leu Pro Lys Arg His Tyr Tyr Lys Gln Lys Lys Phe
355 360 365

Lys Tyr Ala Glu Asp Met Gly Pro Gly Glu Gly Glu His Asp Pro Val
370 375 380

Ala Thr Ser Ser Thr Leu Gly Leu Gln Glu Asn Met Arg Thr Ser
385 390 395

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1997 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: rat P2x clone 3

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:101..1264

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
CGCAGCGAGC CTGCCGGAGC TGGTGGGTGG AGCTACGACC GGGAGCCGAC GGTGGCGAGG      60

GGACCCACAG TGTCCAAGGC GCGGAGCGGT CGGCGGAGCC ATG GCG GGC TGC TGC      115
                                            Met Ala Gly Cys Cys
                                            400

TCC GTG CTC GGG TCC TTC CTG TTC GAG TAC GAC ACG CCG CGC ATC GTG      163
Ser Val Leu Gly Ser Phe Leu Phe Glu Tyr Asp Thr Pro Arg Ile Val
405                 410                 415                 420

CTC ATC CGC AGC CGT AAA GTG GGG CTC ATG AAC CGC GCG GTG CAG CTG      211
Leu Ile Arg Ser Arg Lys Val Gly Leu Met Asn Arg Ala Val Gln Leu
                425                 430                 435

CTC ATC CTG GCT TAC GTC ATC GGG TGG GTG TTC GTG TGG GAA AAG GGC      259
Leu Ile Leu Ala Tyr Val Ile Gly Trp Val Phe Val Trp Glu Lys Gly
            440                 445                 450

TAC CAG GAA ACG GAC TCC GTG GTC AGC TCG GTG ACA ACC AAA GCC AAA      307
Tyr Gln Glu Thr Asp Ser Val Val Ser Ser Val Thr Thr Lys Ala Lys
        455                 460                 465

GGT GTG GCT GTG ACC AAC ACC TCT CAG CTT GGA TTC CGG ATC TGG GAC      355
Gly Val Ala Val Thr Asn Thr Ser Gln Leu Gly Phe Arg Ile Trp Asp
    470                 475                 480

GTG GCG GAC TAT GTG ATT CCA GCT CAG GAG GAA AAC TCC CTC TTC ATT      403
Val Ala Asp Tyr Val Ile Pro Ala Gln Glu Glu Asn Ser Leu Phe Ile
485                 490                 495                 500

ATG ACC AAC ATG ATT GTC ACC GTG AAC CAG ACA CAG AGC ACC TGT CCA      451
Met Thr Asn Met Ile Val Thr Val Asn Gln Thr Gln Ser Thr Cys Pro
                505                 510                 515

GAG ATT CCT GAT AAG ACC AGC ATT TGT AAT TCA GAC GCC GAC TGC ACT      499
Glu Ile Pro Asp Lys Thr Ser Ile Cys Asn Ser Asp Ala Asp Cys Thr
            520                 525                 530
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CCT GGC TCC GTG GAC ACC CAC AGC AGT GGA GTT GCG ACT GGA AGA TGT      547
Pro Gly Ser Val Asp Thr His Ser Ser Gly Val Ala Thr Gly Arg Cys
        535                 540                 545

GTT CCT TTC AAT GAG TCT GTG AAG ACC TGT GAG GTG GCT GCA TGG TGC      595
Val Pro Phe Asn Glu Ser Val Lys Thr Cys Glu Val Ala Ala Trp Cys
    550                 555                 560

CCG GTG GAG AAC GAC GTT GGC GTG CCA ACG CCG GCT TTC TTA AAG GCT      643
Pro Val Glu Asn Asp Val Gly Val Pro Thr Pro Ala Phe Leu Lys Ala
565                 570                 575                 580

GCA GAA AAC TTC ACC CTC TTG GTA AAG AAC AAC ATC TGG TAC CCC AAG      691
Ala Glu Asn Phe Thr Leu Leu Val Lys Asn Asn Ile Trp Tyr Pro Lys
                585                 590                 595

TTT AAC TTC AGC AAG AGG AAC ATC CTC CCC AAC ATC ACC ACG TCC TAC      739
Phe Asn Phe Ser Lys Arg Asn Ile Leu Pro Asn Ile Thr Thr Ser Tyr
            600                 605                 610

CTC AAA TCG TGC ATT TAC AAT GCT CAA ACG GAT CCC TTC TGC CCC ATA      787
Leu Lys Ser Cys Ile Tyr Asn Ala Gln Thr Asp Pro Phe Cys Pro Ile
        615                 620                 625

TTC CGT CTT GGC ACA ATC GTG GGG GAC GCG GGA CAT AGC TTC CAG GAG      835
Phe Arg Leu Gly Thr Ile Val Gly Asp Ala Gly His Ser Phe Gln Glu
    630                 635                 640

ATG GCA GTT GAG GGA GGC ATC ATG GGT ATC CAG ATC AAG TGG GAC TGC      883
Met Ala Val Glu Gly Gly Ile Met Gly Ile Gln Ile Lys Trp Asp Cys
645                 650                 655                 660

AAC CTG GAT AGA GCC GCC TCC CTT TGC CTG CCC AGA TAT TCC TTC CGG      931
Asn Leu Asp Arg Ala Ala Ser Leu Cys Leu Pro Arg Tyr Ser Phe Arg
                665                 670                 675

CGC CTG GAC ACC CGG GAC CTG GAA CAC AAT GTG TCT CCT GGC TAC AAT      979
Arg Leu Asp Thr Arg Asp Leu Glu His Asn Val Ser Pro Gly Tyr Asn
            680                 685                 690

TTC AGG TTT GCC AAG TAC TAC AGG GAC CTG GCC GGC AAA GAG CAG CGC     1027
Phe Arg Phe Ala Lys Tyr Tyr Arg Asp Leu Ala Gly Lys Glu Gln Arg
        695                 700                 705

ACA CTC ACC AAG GCG TAC GGC ATC CGC TTT GAC ATC ATC GTG TTT GGA     1075
Thr Leu Thr Lys Ala Tyr Gly Ile Arg Phe Asp Ile Ile Val Phe Gly
    710                 715                 720

AAG GCT GGG AAG TTT GAC ATC ATC CCT ACC ATG ATC AAC GTT GGC TCT     1123
Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Ile Asn Val Gly Ser
725                 730                 735                 740

GGC TTG GCG CTC CTC GGG GTG GCG ACG GTG CTC TGT GAC GTC ATA GTC     1171
Gly Leu Ala Leu Leu Gly Val Ala Thr Val Leu Cys Asp Val Ile Val
                745                 750                 755

CTC TAC TGC ATG AAG AAG AAA TAC TAC TAC CGG GAC AAG AAA TAT AAG     1219
Leu Tyr Cys Met Lys Lys Lys Tyr Tyr Tyr Arg Asp Lys Lys Tyr Lys
            760                 765                 770
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TAT GTG GAA GAC TAC GAG CAG GGT CTT TCG GGG GAG ATG AAC CAG 1264
Tyr Val Glu Asp Tyr Glu Gln Gly Leu Ser Gly Glu Met Asn Gln
775 780 785

TGACGCCTAA AGTTACATTT CCACCCCGCT CAGCCCGCGA AGCAGAAAGA TGGGGAGAGA 1324

TGGCTACTGC GTCTGTCACT CTAGAGAAAG CTCCAGAGTT TCAGCTCAGT TCTCCACTCC 1384

ACAAATACTC AGGGTTGCCA AGCACATCTT GTTGGAGCCC GGCTCTTGCT CTGCTGCTCA 1444

GATGGGCTTC CAGATACAAG AATCCTCCTG CTTCTGCCTC TAGGAATGCT GGGATCAAAC 1504

ATGTCACTTG CAATGCCCAT TTCCCATGGG GAGTTTGGCA TTTTTTACAT TTTACCCTTT 1564

CCTTTTGTAT ACATCTAAGG CTGCCCTCAG ACGCAAGACG TTCTTCCACC CTATACACCC 1624

TTTTAATCTC ACTGTGTGTG GGAGGGGGGT CGTTTGCACA CGACGCACGG TGGATGTCTG 1684

GTGTGCTGTT GGCTGGGCCA CCTGTGGCTT ATACAGTGTG AGCGTATGGA GGTAGGAAGG 1744

GTCTGAGAGC AGAGACACTG CTGTGGCTTA CGGACAGGCC CAGGCTCTGT CCACGCACTT 1804

TATTTCTAAG GAAGGAGGCT CTCTCAGGTG CTGTCAGCAG GCCTGGGACA CCATTCCTCT 1864

TCCCTATAAT CAGAGAAGTT GTCCTTGTAG CAAAGGCAGG GTTAGCTTTT CCTTTTATAA 1924

GGGCTGTGTT GAAATGACCT AGGACCAAAC ATTAAAAGAA ATAATTTTTT AAAAAAAAAA 1984

AAAAAAAAAA AAA 1997

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 388 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Ala Gly Cys Cys Ser Val Leu Gly Ser Phe Leu Phe Glu Tyr Asp
1 5 10 15

Thr Pro Arg Ile Val Leu Ile Arg Ser Arg Lys Val Gly Leu Met Asn
20 25 30

Arg Ala Val Gln Leu Leu Ile Leu Ala Tyr Val Ile Gly Trp Val Phe
35 40 45

Val Trp Glu Lys Gly Tyr Gln Glu Thr Asp Ser Val Val Ser Ser Val
50 55 60

Thr Thr Lys Ala Lys Gly Val Ala Val Thr Asn Thr Ser Gln Leu Gly
65 70 75 80

Phe Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln Glu Glu
85 90 95

Asn Ser Leu Phe Ile Met Thr Asn Met Ile Val Thr Val Asn Gln Thr
100 105 110

Gln Ser Thr Cys Pro Glu Ile Pro Asp Lys Thr Ser Ile Cys Asn Ser
115 120 125

Asp Ala Asp Cys Thr Pro Gly Ser Val Asp Thr His Ser Ser Gly Val
130 135 140

Ala Thr Gly Arg Cys Val Pro Phe Asn Glu Ser Val Lys Thr Cys Glu
145 150 155 160

Val Ala Ala Trp Cys Pro Val Glu Asn Asp Val Gly Val Pro Thr Pro
165 170 175

Ala Phe Leu Lys Ala Ala Glu Asn Phe Thr Leu Leu Val Lys Asn Asn
180 185 190

Ile Trp Tyr Pro Lys Phe Asn Phe Ser Lys Arg Asn Ile Leu Pro Asn
195 200 205

Ile Thr Thr Ser Tyr Leu Lys Ser Cys Ile Tyr Asn Ala Gln Thr Asp
210 215 220

Pro Phe Cys Pro Ile Phe Arg Leu Gly Thr Ile Val Gly Asp Ala Gly
225 230 235 240

His Ser Phe Gln Glu Met Ala Val Glu Gly Gly Ile Met Gly Ile Gln
245 250 255

Ile Lys Trp Asp Cys Asn Leu Asp Arg Ala Ala Ser Leu Cys Leu Pro
260 265 270

Arg Tyr Ser Phe Arg Arg Leu Asp Thr Arg Asp Leu Glu His Asn Val
275 280 285

Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Arg Asp Leu Ala
290 295 300

Gly Lys Glu Gln Arg Thr Leu Thr Lys Ala Tyr Gly Ile Arg Phe Asp
305 310 315 320

Ile Ile Val Phe Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met
325 330 335

Ile Asn Val Gly Ser Gly Leu Ala Leu Leu Gly Val Ala Thr Val Leu
340 345 350

Cys Asp Val Ile Val Leu Tyr Cys Met Lys Lys Lys Tyr Tyr Tyr Arg
355 360 365

Asp Lys Lys Tyr Lys Tyr Val Glu Asp Tyr Glu Gln Gly Leu Ser Gly
370 375 380

Glu Met Asn Gln
385

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: rat P2x clone 6

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:163..1353

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
CACTGGGCTA CAGTTGCCTG GCTTACAGGA ACTGGCTCTT TTCCTCAAGC CTCATTAAGC      60

AGCCCACTCC AGTTCTTGAT CTTTGTCTCC CAGTCCTGAA GTCCTTTCTC TCCTTAGGCT     120

GCATCCACAG CCCTTCTAAG TGGCTGTGAG CAGTTTCTCA GT ATG AAC TGT ATA         174
                                               Met Asn Cys Ile
                                                   390

TCA GAC TTC TTC ACC TAC GAG ACT ACC AAG TCG GTG GTT GTG AAG AGC         222
Ser Asp Phe Phe Thr Tyr Glu Thr Thr Lys Ser Val Val Val Lys Ser
        395                 400                 405

TGG ACC ATT GGG ATC ATC AAC CGA GCC GTC CAG CTG CTG ATT ATC TCC         270
Trp Thr Ile Gly Ile Ile Asn Arg Ala Val Gln Leu Leu Ile Ile Ser
    410                 415                 420

TAC TTT GTG GGG TGG GTT TTC TTG CAT GAG AAG GCC TAC CAA GTG AGG         318
Tyr Phe Val Gly Trp Val Phe Leu His Glu Lys Ala Tyr Gln Val Arg
425                 430                 435                 440

GAC ACC GCC ATT GAG TCC TCA GTA GTT ACA AAG GTG AAA GGC TTC GGG         366
Asp Thr Ala Ile Glu Ser Ser Val Val Thr Lys Val Lys Gly Phe Gly
                445                 450                 455

CGC TAT GCC AAC AGA GTC ATG GAC GTG TCG GAT TAT GTG ACC CCA CCC         414
Arg Tyr Ala Asn Arg Val Met Asp Val Ser Asp Tyr Val Thr Pro Pro
            460                 465                 470

CAG GGC ACC TCT GTC TTT GTC ATC ATC ACC AAA ATG ATC GTT ACT GAA         462
Gln Gly Thr Ser Val Phe Val Ile Ile Thr Lys Met Ile Val Thr Glu
        475                 480                 485

AAT CAA ATG CAA GGA TTC TGT CCA GAG AAT GAA GAG AAG TAC CGC TGT         510
Asn Gln Met Gln Gly Phe Cys Pro Glu Asn Glu Glu Lys Tyr Arg Cys
    490                 495                 500

GTG TCT GAC AGC CAG TGT GGG CCT GAA CGC TTC CCA GGT GGG GGG ATC         558
Val Ser Asp Ser Gln Cys Gly Pro Glu Arg Phe Pro Gly Gly Gly Ile
505                 510                 515                 520
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CTC ACC GGC CGC TGC GTG AAC TAC AGC TCT GTT CTC CGG ACC TGT GAG      606
Leu Thr Gly Arg Cys Val Asn Tyr Ser Ser Val Leu Arg Thr Cys Glu
                525                 530                 535

ATC CAG GGC TGG TGC CCC ACT GAG GTG GAC ACC GTG GAG ATG CCT ATC      654
Ile Gln Gly Trp Cys Pro Thr Glu Val Asp Thr Val Glu Met Pro Ile
            540                 545                 550

ATG ATG GAG GCT GAG AAC TTC ACC ATT TTC ATC AAG AAC AGC ATC CGT      702
Met Met Glu Ala Glu Asn Phe Thr Ile Phe Ile Lys Asn Ser Ile Arg
        555                 560                 565

TTC CCT CTC TTC AAC TTT GAG AAG GGA AAC CTC CTG CCT AAC CTC ACC      750
Phe Pro Leu Phe Asn Phe Glu Lys Gly Asn Leu Leu Pro Asn Leu Thr
    570                 575                 580

GAC AAG GAC ATA AAG AGG TGC CGC TTC CAC CCT GAA AAG GCC CCA TTT      798
Asp Lys Asp Ile Lys Arg Cys Arg Phe His Pro Glu Lys Ala Pro Phe
585                 590                 595                 600

TGC CCC ATC TTG AGG GTA GGG GAT GTG GTT AAG TTT GCT GGA CAG GAT      846
Cys Pro Ile Leu Arg Val Gly Asp Val Val Lys Phe Ala Gly Gln Asp
                605                 610                 615

TTT GCC AAG CTG GCC CGC ACG GGT GGC GTT CTG GGT ATT AAG ATC GGC      894
Phe Ala Lys Leu Ala Arg Thr Gly Gly Val Leu Gly Ile Lys Ile Gly
            620                 625                 630

TGG GTG TGC GAT CTA GAC AAG GCC TGG GAC CAG TGC ATC CCT AAA TAT      942
Trp Val Cys Asp Leu Asp Lys Ala Trp Asp Gln Cys Ile Pro Lys Tyr
        635                 640                 645

TCC TTC ACT CGG CTG GAT GGA GTT TCT GAG AAA AGC AGT GTT TCC CCT      990
Ser Phe Thr Arg Leu Asp Gly Val Ser Glu Lys Ser Ser Val Ser Pro
    650                 655                 660

GGC TAC AAC TTC AGG TTT GCC AAA TAC TAT AAG ATG GAG AAC GGC AGC     1038
Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Lys Met Glu Asn Gly Ser
665                 670                 675                 680

GAG TAC CGC ACA CTC CTG AAG GCT TTT GGC ATC CGC TTT GAT GTG CTG     1086
Glu Tyr Arg Thr Leu Leu Lys Ala Phe Gly Ile Arg Phe Asp Val Leu
                685                 690                 695

GTA TAT GGG AAC GCT GGC AAG TTC AAC ATC ATC CCC ACC ATT ATC AGC     1134
Val Tyr Gly Asn Ala Gly Lys Phe Asn Ile Ile Pro Thr Ile Ile Ser
            700                 705                 710

TCG GTG GCG GCC TTC ACT TCT GTG GGA GTG GGC ACT GTT CTC TGT GAC     1182
Ser Val Ala Ala Phe Thr Ser Val Gly Val Gly Thr Val Leu Cys Asp
        715                 720                 725

ATC ATC CTG CTC AAT TTC CTC AAA GGG GCT GAT CAC TAC AAA GCC AGG     1230
Ile Ile Leu Leu Asn Phe Leu Lys Gly Ala Asp His Tyr Lys Ala Arg
    730                 735                 740

AAG TTT GAG GAG GTG ACT GAG ACA ACA CTG AAG GGT ACT GCG TCA ACC     1278
Lys Phe Glu Glu Val Thr Glu Thr Thr Leu Lys Gly Thr Ala Ser Thr
745                 750                 755                 760
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AAC CCA GTG TTC GCC AGT GAC CAG GCC ACT GTG GAG AAG CAG TCT ACA      1326
Asn Pro Val Phe Ala Ser Asp Gln Ala Thr Val Glu Lys Gln Ser Thr
                765                 770                 775

GAC TCA GGG GCC TAT TCT ATT GGT CAC TAGGGCCTCT TCCCAGGGTT            1373
Asp Ser Gly Ala Tyr Ser Ile Gly His
            780                 785

CCATGCTCAC CCTTAGGCTG CAGAACCTGC AAACAGGCCA CTCTATCTAA GCAGTCAGGG    1433

GTGGGAGGGG GAGAAGAAGG GCTGCTATTT CTGCTGTTCA CCCCAAAGAC TAGATCCAGA    1493

TATCTAGGCC CTCACTGTTC AACAGATAGG CAATGCTTCC CACTAAGACT TGAATCTTGC    1553

CTTTACCCCT TGCATGCCTC CCACCTGCTT CCCTGGATCC CAGGACAGCA GCATCCACCC    1613

CTTTCCAAAG GATTGAGAAA ATGGTAGCTA AGGTTACACC CATAGGACCT ACCACGTACC    1673

AAGCACTTCC ACACATATTA TCCCTTTTCA CCCTTAAAAT AATCCTATAA GGTAGAAAAA    1733

AAAAAAAAAA AAAAAAAAAA                                                1753
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Asn Cys Ile Ser Asp Phe Phe Thr Tyr Glu Thr Thr Lys Ser Val
1 5 10 15

Val Val Lys Ser Trp Thr Ile Gly Ile Ile Asn Arg Ala Val Gln Leu
20 25 30

Leu Ile Ile Ser Tyr Phe Val Gly Trp Val Phe Leu His Glu Lys Ala
35 40 45

Tyr Gln Val Arg Asp Thr Ala Ile Glu Ser Ser Val Val Thr Lys Val
50 55 60

Lys Gly Phe Gly Arg Tyr Ala Asn Arg Val Met Asp Val Ser Asp Tyr
65 70 75 80

Val Thr Pro Pro Gln Gly Thr Ser Val Phe Val Ile Ile Thr Lys Met
85 90 95

Ile Val Thr Glu Asn Gln Met Gln Gly Phe Cys Pro Glu Asn Glu Glu
100 105 110

Lys Tyr Arg Cys Val Ser Asp Ser Gln Cys Gly Pro Glu Arg Phe Pro
115 120 125

Gly Gly Gly Ile Leu Thr Gly Arg Cys Val Asn Tyr Ser Ser Val Leu
130 135 140

Arg Thr Cys Glu Ile Gln Gly Trp Cys Pro Thr Glu Val Asp Thr Val
145 150 155 160

Glu Met Pro Ile Met Met Glu Ala Glu Asn Phe Thr Ile Phe Ile Lys
165 170 175

Asn Ser Ile Arg Phe Pro Leu Phe Asn Phe Glu Lys Gly Asn Leu Leu
180 185 190

Pro Asn Leu Thr Asp Lys Asp Ile Lys Arg Cys Arg Phe His Pro Glu
195 200 205

Lys Ala Pro Phe Cys Pro Ile Leu Arg Val Gly Asp Val Val Lys Phe
210 215 220

Ala Gly Gln Asp Phe Ala Lys Leu Ala Arg Thr Gly Gly Val Leu Gly
225 230 235 240

Ile Lys Ile Gly Trp Val Cys Asp Leu Asp Lys Ala Trp Asp Gln Cys
245 250 255

Ile Pro Lys Tyr Ser Phe Thr Arg Leu Asp Gly Val Ser Glu Lys Ser
260 265 270

Ser Val Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Lys Met
275 280 285

Glu Asn Gly Ser Glu Tyr Arg Thr Leu Leu Lys Ala Phe Gly Ile Arg
290 295 300

Phe Asp Val Leu Val Tyr Gly Asn Ala Gly Lys Phe Asn Ile Ile Pro
305 310 315 320

Thr Ile Ile Ser Ser Val Ala Ala Phe Thr Ser Val Gly Val Gly Thr
325 330 335

Val Leu Cys Asp Ile Ile Leu Leu Asn Phe Leu Lys Gly Ala Asp His
340 345 350

Tyr Lys Ala Arg Lys Phe Glu Glu Val Thr Glu Thr Thr Leu Lys Gly
355 360 365

Thr Ala Ser Thr Asn Pro Val Phe Ala Ser Asp Gln Ala Thr Val Glu
370 375 380

Lys Gln Ser Thr Asp Ser Gly Ala Tyr Ser Ile Gly His
385 390 395

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2643 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: human P2x

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:174..1370

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
GCCTCCAGCT GACCTCTGGC TCCTGTCCTC TGGCTCCACC TGCACCGCCC TGCTCTTCCT      60

AAGGGGCCAG GAAGCCCCCA GAAGCTCTAC CATCGACGTG GGTGGTGGCA CCCGGCTCAC     120

CCTGAGAGCA GAGGGCGTGC AGGGGGCTCA GTTCTGAGCC CAGCCGGCCC ACC ATG        176
                                                           Met

GCA CGG CGG TTC CAG GAG GAG CTG GCC GCC TTC CTC TTC GAG TAT GAC        224
Ala Arg Arg Phe Gln Glu Glu Leu Ala Ala Phe Leu Phe Glu Tyr Asp
    400                 405                 410

ACC CCC CGC ATG GTG CTG GTG CGT AAT AAG AAG GTG GGC GTT ATC TTC        272
Thr Pro Arg Met Val Leu Val Arg Asn Lys Lys Val Gly Val Ile Phe
415                 420                 425                 430

CGA CTG ATC CAG CTG GTG GTC CTG GTC TAC GTC ATC GGG TGG GTG TTT        320
Arg Leu Ile Gln Leu Val Val Leu Val Tyr Val Ile Gly Trp Val Phe
                435                 440                 445

CTC TAT GAG AAG GGC TAC CAG ACC TCG AGC GGC CTC ATC AGC AGT GTC        368
Leu Tyr Glu Lys Gly Tyr Gln Thr Ser Ser Gly Leu Ile Ser Ser Val
            450                 455                 460

TCT GTG AAA CTC AAG GGC CTG GCC GTG ACC CAG CTC CCT GGC CTC GGC        416
Ser Val Lys Leu Lys Gly Leu Ala Val Thr Gln Leu Pro Gly Leu Gly
        465                 470                 475

CCC CAG GTC TGG GAT GTG GCT GAC TAC GTC TTC CCA GCC CAG GGG GAC        464
Pro Gln Val Trp Asp Val Ala Asp Tyr Val Phe Pro Ala Gln Gly Asp
    480                 485                 490

AAC TCC TTC GTG GTC ATG ACC AAT TTC ATC GTG ACC CCG AAG CAG ACT        512
Asn Ser Phe Val Val Met Thr Asn Phe Ile Val Thr Pro Lys Gln Thr
495                 500                 505                 510
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CAA GGC TAC TGC GCA GAG CAC CCA GAA GGG GGC ATA TGC AAG GAA GAC      560
Gln Gly Tyr Cys Ala Glu His Pro Glu Gly Gly Ile Cys Lys Glu Asp
                515                 520                 525

AGT GGC TGT ACC CCT GGG AAG GCC AAG AGG AAG GCC CAA GGC ATC CGC      608
Ser Gly Cys Thr Pro Gly Lys Ala Lys Arg Lys Ala Gln Gly Ile Arg
            530                 535                 540

ACG GGC AAG TGT GTG GCC TTC AAC GAC ACT GTG AAG ACG TGT GAG ATC      656
Thr Gly Lys Cys Val Ala Phe Asn Asp Thr Val Lys Thr Cys Glu Ile
        545                 550                 555

TTT GGC TGG TGC CCC GTG GAG GTG GAT GAC GAC ATC CCG CGC CCT GCC      704
Phe Gly Trp Cys Pro Val Glu Val Asp Asp Asp Ile Pro Arg Pro Ala
    560                 565                 570

CTT CTC CGA GAG GCC GAG AAC TTC ACT CTT TTC ATC AAG AAC AGC ATC      752
Leu Leu Arg Glu Ala Glu Asn Phe Thr Leu Phe Ile Lys Asn Ser Ile
575                 580                 585                 590

AGC TTT CCA CGC TTC AAG GTC AAC AGG CGC AAC CTG GTG GAG GAG GTG      800
Ser Phe Pro Arg Phe Lys Val Asn Arg Arg Asn Leu Val Glu Glu Val
                595                 600                 605

AAT GCT GCC CAC ATG AAG ACC TGC CTC TTT CAC AAG ACC CTG CAC CCC      848
Asn Ala Ala His Met Lys Thr Cys Leu Phe His Lys Thr Leu His Pro
            610                 615                 620

CTG TGC CCA GTC TTC CAG CTT GGC TAC GTG GTG CAA GAG TCA GGC CAG      896
Leu Cys Pro Val Phe Gln Leu Gly Tyr Val Val Gln Glu Ser Gly Gln
        625                 630                 635

AAC TTC AGC ACC CTG GCT GAG AAG GGT GGA GTG GTT GGC ATC ACC ATC      944
Asn Phe Ser Thr Leu Ala Glu Lys Gly Gly Val Val Gly Ile Thr Ile
    640                 645                 650

GAC TGG CAC TGT GAC CTG GAC TGG CAC GTA CGG CAC TGC AGA CCC ATC      992
Asp Trp His Cys Asp Leu Asp Trp His Val Arg His Cys Arg Pro Ile
655                 660                 665                 670

TAT GAG TTC CAT GGG CTG TAC GAA GAG AAA AAT CTC TCC CCA GGC TTC     1040
Tyr Glu Phe His Gly Leu Tyr Glu Glu Lys Asn Leu Ser Pro Gly Phe
                675                 680                 685

AAC TTC AGG TTT GCC AGG CAC TTT GTG GAG AAC GGG ACC AAC TAC CGT     1088
Asn Phe Arg Phe Ala Arg His Phe Val Glu Asn Gly Thr Asn Tyr Arg
            690                 695                 700

CAC CTC TTC AAG GTG TTT GGG ATT CGC TTT GAC ATC CTG GTG GAC GGC     1136
His Leu Phe Lys Val Phe Gly Ile Arg Phe Asp Ile Leu Val Asp Gly
        705                 710                 715

AAG GCC GGG AAG TTT GAC ATC ATC CCT ACA ATG ACC ACC ATC GGC TCT     1184
Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Thr Thr Ile Gly Ser
    720                 725                 730

GGA ATT GGC ATC TTT GGG GTG GCC ACA GTT CTC TGT GAC CTG CTG CTG     1232
Gly Ile Gly Ile Phe Gly Val Ala Thr Val Leu Cys Asp Leu Leu Leu
735                 740                 745                 750
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CTT CAC ATC CTG CCT AAG AGG CAC TAC TAC AAG CAG AAG AAG TTC AAA 1280
Leu His Ile Leu Pro Lys Arg His Tyr Tyr Lys Gln Lys Lys Phe Lys
755 760 765

TAC GCT GAG GAC ATG GGG CCA GGG GCG GCT GAG CGT GAC CTC GCA GCT 1328
Tyr Ala Glu Asp Met Gly Pro Gly Ala Ala Glu Arg Asp Leu Ala Ala
770 775 780

ACC AGC TCC ACC CTG GGC CTG CAG GAG AAC ATG AGG ACA TCC 1370
Thr Ser Ser Thr Leu Gly Leu Gln Glu Asn Met Arg Thr Ser
785 790 795

TGATGCTCGG GCCCCAACTC CTGACTGGGT GCAGCGTGAG GCTTCAGCCT GGAGCCCTGG 1430

TGGGTCCCAG CCAGGGCAGA GGGGCCTCCC CAGGAAGTCT CCTACCCTCT CAGCCAGGCA 1490

GAGAGCAGTT TGCCAGAAGC TCAGGGTGCA TAGTAGGAGA GACCTGTGCA AATCTGAGCT 1550

CCGGCTCCGA CCCCACACAC CCTGAGGGAG GCCTACCCTA GCCTCAGCCG CTCCTGGTGG 1610

GGGAATGGCT GGGGGTTGGG CAGGACCCTC CCACACACCT GCACCCTAGC TTCGTGCTTC 1670

TCTCTCCGGA CTCTCATTAT CCAACCCGCT GCCTCCATTT CTCTAGATCT GTGCTCTCCG 1730

ATGTGGCAGT CAGTAACCAT AGGTGACTAA ATTAAACTAA AATAAAATAG AATGAAACAC 1790

AAAATTCAAT TCCTCGGCTG AACTAGCCAC ATTTCAACTG CTCAGTAGAT ACGTGTGGTT 1850

AGTGGCTGCC ATACTGGACA GCTCGGGGCA TTTTCACTGT CAAAGAAAGT TCTATTAGAC 1910

AGCCCTGCTT GAGCCCTGTT TCTTCCTGGC TTCGGTTTCC CTGGGGAACT TATCGACAAT 1970

GCAAGCTCCT GGGCCCACCC CCAGACCTCC TGAACCAAAA GCTCCAGGGC TGGCCGTATG 2030

ATCTGTGTGG ATGGCAAACT CCCCAGGCCA TTCTGGGACC TAAGTTTAAG AAGTGCCGTC 2090

CTCGAACTTT CTGACTCTAA GCTCCTGAGC GGGAGTCAGA CTTAGCCCTG AGCCTGCACT 2150

TCCTGTTCAG GTGCAGACAC TGAACAGGGT CTCAAACACC TTCAGCATGT GTGTTGTGTG 2210

CTCACGTGCC ACACAGTGTC TCATGCACAC AACCCAGTGT ACACACCACC TACGTGCACA 2270

CAGCATCCTT CCACACTGTG TATGTGAACA GCTTGGGCCC TGCAAACACA ACCATCTACA 2330

CACATCTACA CCCCCAAGCA CACACACATG GTCCGTGCCA TGTCACCTCC ATAGGGAAAG 2390

GCTTCTCTCC AAGTGTGCCA GGCCAGGACA GCCCTCCCAG CCATGAATCC TTACTCAGCT 2450

ACCTCGGGTT GGGGTGGGAG CCCCAGCCAA ATCCTGGGCT CCCTGCCTGT GGCTCAGCCC 2510

CAGCTCCCAA GGCCTGCCTG GCTCTGTCTG AACAGAAGGT CTGGGGGAAG CGAGGGGTGG 2570

AGTACAATAA AGGGAATGAG GACAAACAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 2630

AAAAAAAAAA AAA 2643

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Met Ala Arg Arg Phe Gln Glu Glu Leu Ala Ala Phe Leu Phe Glu Tyr
 1               5                  10                  15

Asp Thr Pro Arg Met Val Leu Val Arg Asn Lys Lys Val Gly Val Ile
            20                  25                  30

Phe Arg Leu Ile Gln Leu Val Val Leu Val Tyr Val Ile Gly Trp Val
        35                  40                  45

Phe Leu Tyr Glu Lys Gly Tyr Gln Thr Ser Ser Gly Leu Ile Ser Ser
    50                  55                  60

Val Ser Val Lys Leu Lys Gly Leu Ala Val Thr Gln Leu Pro Gly Leu
65                  70                  75                  80

Gly Pro Gln Val Trp Asp Val Ala Asp Tyr Val Phe Pro Ala Gln Gly
                85                  90                  95

Asp Asn Ser Phe Val Val Met Thr Asn Phe Ile Val Thr Pro Lys Gln
            100                 105                 110

Thr Gln Gly Tyr Cys Ala Glu His Pro Glu Gly Gly Ile Cys Lys Glu
        115                 120                 125

Asp Ser Gly Cys Thr Pro Gly Lys Ala Lys Arg Lys Ala Gln Gly Ile
    130                 135                 140

Arg Thr Gly Lys Cys Val Ala Phe Asn Asp Thr Val Lys Thr Cys Glu
145                 150                 155                 160

Ile Phe Gly Trp Cys Pro Val Glu Val Asp Asp Ile Pro Arg Pro
                165                 170                 175

Ala Leu Leu Arg Glu Ala Glu Asn Phe Thr Leu Phe Ile Lys Asn Ser
            180                 185                 190

Ile Ser Phe Pro Arg Phe Lys Val Asn Arg Arg Asn Leu Val Glu Glu
        195                 200                 205

Val Asn Ala Ala His Met Lys Thr Cys Leu Phe His Lys Thr Leu His
    210                 215                 220

Pro Leu Cys Pro Val Phe Gln Leu Gly Tyr Val Val Gln Glu Ser Gly
225                 230                 235                 240

Gln Asn Phe Ser Thr Leu Ala Glu Lys Gly Gly Val Val Gly Ile Thr
                245                 250                 255

Ile Asp Trp His Cys Asp Leu Asp Trp His Val Arg His Cys Arg Pro
            260                 265                 270
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Ile Tyr Glu Phe His Gly Leu Tyr Glu Glu Lys Asn Leu Ser Pro Gly
275 280 285

Phe Asn Phe Arg Phe Ala Arg His Phe Val Glu Asn Gly Thr Asn Tyr
290 295 300

Arg His Leu Phe Lys Val Phe Gly Ile Arg Phe Asp Ile Leu Val Asp
305 310 315 320

Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Thr Thr Ile Gly
325 330 335

Ser Gly Ile Gly Ile Phe Gly Val Ala Thr Val Leu Cys Asp Leu Leu
340 345 350

Leu Leu His Ile Leu Pro Lys Arg His Tyr Tyr Lys Gln Lys Lys Phe
355 360 365

Lys Tyr Ala Glu Asp Met Gly Pro Gly Ala Ala Glu Arg Asp Leu Ala
370 375 380

Ala Thr Ser Ser Thr Leu Gly Leu Gln Glu Asn Met Arg Thr Ser
385 390 395

CLAIMS

1. A recombinant or isolated DNA molecule encoding a P_{2X} receptor, wherein the receptor:

(a) has the amino sequence shown in Figure 1, Figure 2, Figure 3 or Figure 4; or

(b) is substantially homologous to the sequence shown in Figure 1, Figure 2, Figure 3 or Figure 4;

or a fragment of such a DNA molecule, which fragment includes at least 15 nucleotides taken from nucleotides 1 to 813 shown in Figure 1, from the full nucleotide sequences shown in Figures 2 and 3, or from nucleotides 1 to 1744 shown in Figure 4.

2. A recombinant or isolated DNA molecule encoding a P_{2X} receptor, wherein the receptor:

(a) has the amino sequence shown in Figure 1 or Figure 4; or

(b) is substantially homologous to the sequence shown in Figure 1 or Figure 4;

or a fragment of such a DNA molecule, which fragment includes at least 15 nucleotides taken from nucleotides 1 to 813 shown in Figure 1 or from nucleotides 1 to 777 shown in Figure 4.

3. A recombinant or isolated DNA molecule encoding a P_{2X} receptor, wherein the receptor:

(a) has the amino sequence shown in Figure 1; or

(b) is substantially homologous to the sequence shown in Figure 1;

or a fragment of such a DNA molecule, which fragment includes at least 15 nucleotides taken from nucleotides 1 to 813 shown in Figure 1.

4. A DNA molecule as claimed in any of claims 1 to 3, which encodes a human P_{2X} receptor.

5. A DNA molecule as claimed in any of claims 1 to 4, which is cDNA.

6. A DNA molecule as claimed in any of claims 1 to 5, which is in the form of a vector.

7. A host cell transformed or transfected with a vector as described in claim 6.

8. A host cell as claimed in claim 7 which is a stably transfected mammalian cell which expresses a P_{2X} receptor.

9. A preparation of P_{2X} receptor which is free of protein with which it is naturally associated.

10. A preparation of P_{2X} receptor which is free of P_{2Y} receptor.

11. P_{2X} receptor as prepared by recombinant DNA technology.

12. A peptide fragment of P_{2X} receptor which includes an epitope which is immunologically non-cross reactive with the RP-2 polypeptide disclosed in Owens *et al.* (*loc. cit.*).

13. An antibody which is specific for an epitope of P_{2X} receptor which is immunologically non-cross reactive with the RP-2 polypeptide disclosed in Owens *et al.* (*loc. cit.*).

14. An antibody as claimed in claim 13, which is a monoclonal antibody.

15. A cell expressing an antibody as claimed in claim 14.

16. The use of a P_{2X} receptor or a preparation thereof, as claimed in claim 7, 8 or 9, as a screen for compounds useful in the treatment or prophylaxis of a human or non-human animal disease or condition.

17. The use of a P_{2X} receptor or a preparation thereof as claimed in claim 9, 10 or 11 as a screen for identifying a P_{2X} agonist or a P_{2X} antagonist.

18. A P_{2X} agonist or a P_{2X} antagonist identified by a scrren as described in claim 17.

19. A method for obtaining a DNA molecule according to claim 1, wherein the molecule is obtained by chemical synthesis or by using recombinant DNA technology.

20. A method for obtaining a P_{2X} receptor comprising expressing the P_{2X} receptor using a host cell according to claim 8 and, optionallly, purifying the P_{2X} receptor.

21. A DNA molecule, a P_{2X} receptor, a P_{2X} agonist or a P_{2X} antagonist, a method, or a use, substantially as hereinbefore described, with reference to the accompanying examples.

FIGURE 1

P2xα 1 cDNA from rat vas deferens

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  1 gccaaaagctgttctgatcacccagggtttttcctcccaaacccagaccccaccatcgaacctccaactctggtcccacct 80

 81 agcctgctctgtccttaaggggccgggaagccccagtcactccactgctattgtagatgcagatggtggcctgcccttga 160

161 ccatagaggccgtgtggggtgttcatctctgagccccttctggcccacc ATG GCT CGG CGG CTG CAA GAT     230
  1                                                    M   A   R   R   L   Q   D       7

231 GAG CTG TCA GCC TTC TTC TTT GAA TAT GAC ACT CCC CGG ATG GTG CTG GTA CGA AAC AAG     290
  8 E   L   S   A   F   F   F   E   Y   D   T   P   R   M   V   L   V   R   N   K      27

291 AAG GTG GGA GTC ATT TTC CGT CTG ATC CAG TTG GTG GTT CTG GTC TAC GTC ATT GGG TGG     350
 28 K   V   G   V   I   F   R   L   I   Q   L   V   V   L   V   Y   V   I   G   W      47

351 GTG TTT GTC TAT GAA AAA GGA TAC CAG ACC TCA AGT GAC CTC ATC AGC AGT GTG TCC GTG     410
 48 V   F   V   Y   E   K   G   Y   Q   T   S   S   D   L   I   S   S   V   S   V      67

411 AAG CTC AAG GGC TTG GCT GTG ACC CAG CTC CAG GGC CTG GGA CCC CAG GTC TGG GAC GTG     470
 68 K   L   K   G   L   A   V   T   Q   L   Q   G   L   G   P   Q   V   W   D   V      87

471 GCT GAC TAT GTC TTC CCA GCA CAC GGG GAC AGC TCC TTT GTA GTT ATG ACC AAC TTC ATC     530
 88 A   D   Y   V   F   P   A   H   G   D   S   S   F   V   V   M   T   N   F   I     107

531 GTG ACC CCT CAG CAG ACT CAA GGC CAT TGT GCA GAG AAC CCA GAA GGT GGC ATA TGC CAG     590
108 V   T   P   Q   Q   T   Q   G   H   C   A   E   N   P   E   G   G   I   C   Q     127

591 GAT GAC AGT GGC TGC ACT CCA GGA AAA GCA GAA AGG AAA GCC CAA GGT ATT CGC ACA GGC     650
128 D   D   S   G   C   T   P   G   K   A   E   R   K   A   Q   G   I   R   T   G     147

651 AAC TGT GTG CCC TTC AAT GGC ACT GTG AAG ACA TGT GAG ATC TTT GGT TGG TGT CCT GTA     710
148 N   C   V   P   F   N   G   T   V   K   T   C   E   I   F   G   W   C   P   V     167

711 GAG GTG GAT GAC AAG ATC CCA AGC CCT GCT CTT CTT CGT GAG GCT GAG AAC TTC ACC CTC     770
168 E   V   D   D   K   I   P   S   P   A   L   L   R   E   A   E   N   F   T   L     187

        CCC CAG CTG GCA CAT GGC TGC TAC CCA TGC CCT CCA CAC AG
        P   Q   L   A   H   G   C   Y   P   C   P   P   H   R
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sequence of RP-2

FIGURE 1 (cont'd)

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771 TTC ATC AAA AAC AGC ATC AGC TTT CCA CGC TTC AAG GTC AAC AGG CGC AAC CTG GTA GAG  830
188 F   I   K   N   S   I   S   F   P   R   F   K   V   N   R   R   N   L   V   E  207

831 GAG GTG AAC GGC ACC TAC ATG AAG AAG TGC CTC TAT CAC AAG ATT CAA CAC CCC CTG TGC  890
208 E   V   N   G   T   Y   M   K   K   C   L   Y   H   K   I   Q   H   P   L   C  227

891 CCA GTC TTC AAC CTT GGC TAT GTG GTG CGA GAG TCA GGC CAG GAC TTC CGC AGC CTT GCT  950
228 P   V   F   N   L   G   Y   V   V   R   E   S   G   Q   D   F   R   S   L   A  247

951 GAG AAG GGT GGG GTG GTT GGT ATC ACC ATT GAC TGG AAG TGT GAT CTG GAC TGG CAC GTT 1010
248 E   K   G   G   V   V   G   I   T   I   D   W   K   C   D   L   D   W   H   V  267

1011 CGG CAC TGC AAA CCC ATC TAC CAG TTC CAC GGA CTG TAT GGG GAG AAG AAC CTG TCT CCA 1070
268  R   H   C   K   P   I   Y   Q   F   H   G   L   Y   G   E   K   N   L   S   P  287

1071 GGC TTC AAC TTC AGA TTT GCC AGG CAT TTC GTG CAG AAT GGG ACA AAC CGT CGT CAC CTC 1130
288  G   F   N   F   R   F   A   R   H   F   V   Q   N   G   T   N   R   R   H   L  307

1131 TTC AAG GTG TTT GGG ATT CAC TTT GAT ATC CTT GTG GAT GGC AAG GCT GGG AAG TTT GAC 1190
308  F   K   V   F   G   I   H   F   D   I   L   V   D   G   K   A   G   K   F   D  327

1191 ATC ATC CCT ACT ATG ACT ACT ATC GGT TCT GGG ATT GGC ATC TTT GGA GTG GCC ACA GTG 1250
328  I   I   P   T   M   T   T   I   G   S   G   I   G   I   F   G   V   A   T   V  347

1251 CTT TGT GAT CTC TTA TTG CTC CAC ATC CTG CCT AAG AGG CAC TAC TAC AAG CAG AAG AAG 1310
348  L   C   D   L   L   L   L   H   I   L   P   K   R   H   Y   Y   K   Q   K   K  367

1311 TTC AAA TAT GCC GAG GAC ATG GGG CCG GGA GAG GGT GAA CAT GAC CCC GTG GCC ACC AGC 1370
368  F   K   Y   A   E   D   M   G   P   G   E   G   E   H   D   P   V   A   T   S  387

1371 TCC ACT CTG GGC CTG CAG GAG AAC ATG AGG ACC TCC TGA ccttagtcttgagatccggacttgacgc 1437
388  S   T   L   G   L   Q   E   N   M   R   T   S   *                              400

1438 agtgtgtggcttccggcaagggctgatggctttgagccagggcagagggcattcccagaggctttcctgcaaggcagaca 1517

1518 ccagtggccctctggttcagcatgaagacaggcaagactttggatttcagagctctggtttcagttccacatgtcccttc 1597

                                    end of rp-2 sequence -/

1598 ctgagggatgcctcctccagttttcaccaatttgggttcatatggctgggcccctcacacatctatactctagctttgtg 1677

1678 cttaaggctcaggctgtcattgtctttcccacagccttacctgcctagatttgggctcttccacatggtagccactagcc 1757

1758 agatgtgtcagtttgaactttaattaaaatataataaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 1837
```

FIGURE 2

rat P2X clone 3

```
   1 cgcagcgagcctgccggagctggtgggtggagctacgaccgggagccgacggtggcgaggggacccacagtgtccaaggc 80

  81 gcggagcggtcggcggagcc ATG GCG GGC TGC TGC TCC GTG CTC GGG TCC TTC CTG TTC GAG TAC 145
   1                        M   A   G   C   C   S   V   L   G   S   F   L   F   E   Y  15

 146 GAC ACG CCG CGC ATC GTG CTC ATC CGC AGC CGT AAA GTG GGG CTC ATG AAC CGC GCG GTG 205
  16 D   T   P   R   I   V   L   I   R   S   R   K   V   G   L   M   N   R   A   V  35

 206 CAG CTG CTC ATC CTG GCT TAC GTC ATC GGG TGG GTG TTC GTG TGG GAA AAG GGC TAC CAG 265
  36 Q   L   L   I   L   A   Y   V   I   G   W   V   F   V   W   E   K   G   Y   Q  55

 266 GAA ACG GAC TCC GTG GTC AGC TCG GTG ACA ACC AAA GCC AAA GGT GTG GCT GTG ACC AAC 325
  56 E   T   D   S   V   V   S   S   V   T   T   K   A   K   G   V   A   V   T   N  75

 326 ACC TCT CAG CTT GGA TTC CGG ATC TGG GAC GTG GCG GAC TAT GTG ATT CCA GCT CAG GAG 385
  76 T   S   Q   L   G   F   R   I   W   D   V   A   D   Y   V   I   P   A   Q   E  95

 386 GAA AAC TCC CTC TTC ATT ATG ACC AAC ATG ATT GTC ACC GTG AAC CAG ACA CAG AGC ACC 445
  96 E   N   S   L   F   I   M   T   N   M   I   V   T   V   N   Q   T   Q   S   T 115

 446 TGT CCA GAG ATT CCT GAT AAG ACC AGC ATT TGT AAT TCA GAC GCC GAC TGC ACT CCT GGC 505
 116 C   P   E   I   P   D   K   T   S   I   C   N   S   D   A   D   C   T   P   G 135

 506 TCC GTG GAC ACC CAC AGC AGT GGA GTT GCG ACT GGA AGA TGT GTT CCT TTC AAT GAG TCT 565
 136 S   V   D   T   H   S   S   G   V   A   T   G   R   C   V   P   F   N   E   S 155

 566 GTG AAG ACC TGT GAG GTG GCT GCA TGG TGC CCG GTG GAG AAC GAC GTT GGC GTG CCA ACG 625
 156 V   K   T   C   E   V   A   A   W   C   P   V   E   N   D   V   G   V   P   T 175

 626 CCG GCT TTC TTA AAG GCT GCA GAA AAC TTC ACC CTC TTG GTA AAG AAC AAC ATC TGG TAC 685
 176 P   A   F   L   K   A   A   E   N   F   T   L   L   V   K   N   N   I   W   Y 195

 686 CCC AAG TTT AAC TTC AGC AAG AGG AAC ATC CTC CCC AAC ATC ACC ACG TCC TAC CTC AAA 745
 196 P   K   F   N   F   S   K   R   N   I   L   P   N   I   T   T   S   Y   L   K 215

 746 TCG TGC ATT TAC AAT GCT CAA ACG GAT CCC TTC TGC CCC ATA TTC CGT CTT GGC ACA ATC 805
 216 S   C   I   Y   N   A   Q   T   D   P   F   C   P   I   F   R   L   G   T   I 235
```

FIGURE 2 (cont'd)

```
806 GTG GGG GAC GCG GGA CAT AGC TTC CAG GAG ATG GCA GTT GAG GGA GGC ATC ATG GGT ATC  865
236 V   G   D   A   G   H   S   F   Q   E   M   A   V   E   G   G   I   M   G   I    255

866 CAG ATC AAG TGG GAC TGC AAC CTG GAT AGA GCC GCC TCC CTT TGC CTG CCC AGA TAT TCC  925
256 Q   I   K   W   D   C   N   L   D   R   A   A   S   L   C   L   P   R   Y   S    275

926 TTC CGG CGC CTG GAC ACC CGG GAC CTG GAA CAC AAT GTG TCT CCT GGC TAC AAT TTC AGG  985
276 F   R   R   L   D   T   R   D   L   E   H   N   V   S   P   G   Y   N   F   R    295

986 TTT GCC AAG TAC TAC AGG GAC CTG GCC GGC AAA GAG CAG CGC ACA CTC ACC AAG GCG TAC  1045
296 F   A   K   Y   Y   R   D   L   A   G   K   E   Q   R   T   L   T   K   A   Y    315

1046 GGC ATC CGC TTT GAC ATC ATC GTG TTT GGA AAG GCT GGG AAG TTT GAC ATC ATC CCT ACC  1105
316  G   I   R   F   D   I   I   V   F   G   K   A   G   K   F   D   I   I   P   T    335

1106 ATG ATC AAC GTT GGC TCT GGC TTG GCG CTC CTC GGG GTG GCG ACG GTG CTC TGT GAC GTC  1165
336  M   I   N   V   G   S   G   L   A   L   L   G   V   A   T   V   L   C   D   V    355

1166 ATA GTC CTC TAC TGC ATG AAG AAG AAA TAC TAC TAC CGG GAC AAG AAA TAT AAG TAT GTG  1225
356  I   V   L   Y   C   M   K   K   K   Y   Y   Y   R   D   K   K   Y   K   Y   V    375

1226 GAA GAC TAC GAG CAG GGT CTT TCG GGG GAG ATG AAC CAG TGA cgcctaaagttacatttccacccc  1291
376  E   D   Y   E   Q   G   L   S   G   E   M   N   Q   *                            389

1292 gctcagcccgcgaagcagaaagatggggagagatggctactgcgtctgtcactctagagaaagctccagagtttcagctc  1371

1372 agttctccactccacaaatactcagggttgccaagcacatcttgttggagcccggctcttgctctgctgctcagatgggc  1451

1452 ttccagatacaagaatcctcctgcttctgcctctaggaatgctgggatcaaacatgtcacttgcaatgcccatttcccat  1531

1532 ggggagtttggcatttttacattttacccttcctttttgtatacatctaaggctgccctcagacgcaagacgttcttcc  1611

1612 accctatacacccttttaatctcactgtgtgtgggaggggggtcgtttgcacacgacgcacggtggatgtctggtgtgct  1691

1692 gttggctgggccacctgtggcttatacagtgtgagcgtatggaggtaggaagggtctgagagcagagacactgctgtggc  1771

1772 ttacggacaggcccaggctctgtccacgcactttatttctaaggaaggaggctctctcaggtgctgtcagcaggcctggg  1851

1852 acaccattcctcttccctataatcagagaagttgtccttgtagcaaaggcagggttagcttttccttttataagggctgt  1931

1932 gttgaaatgacctaggaccaaacattaaaagaaataatttttttaaaaaaaaaaaaaaaaaaaaaaa              1997
```

FIGURE 3

rat P2X clone 6

```
   1 cactgggctacagttgcctggcttacaggaactggctcttttcctcaagcctcattaagcagcccactccagttcttgat  80

  81 ctttgtctcccagtcctgaagtcctttctctccttaggctgcatccacagcccttctaagtggctgtgagcagtttctca 160

 161 gt ATG AAC TGT ATA TCA GAC TTC TTC ACC TAC GAG ACT ACC AAG TCG GTG GTT GTG AAG   219
   1    M   N   C   I   S   D   F   F   T   Y   E   T   T   K   S   V   V   V   K    19

 220 AGC TGG ACC ATT GGG ATC ATC AAC CGA GCC GTC CAG CTG CTG ATT ATC TCC TAC TTT GTG   279
  20  S   W   T   I   G   I   I   N   R   A   V   Q   L   L   I   I   S   Y   F   V    39

 280 GGG TGG GTT TTC TTG CAT GAG AAG GCC TAC CAA GTG AGG GAC ACC GCC ATT GAG TCC TCA   339
  40  G   W   V   F   L   H   E   K   A   Y   Q   V   R   D   T   A   I   E   S   S    59

 340 GTA GTT ACA AAG GTG AAA GGC TTC GGG CGC TAT GCC AAC AGA GTC ATG GAC GTG TCG GAT   399
  60  V   V   T   K   V   K   G   F   G   R   Y   A   N   R   V   M   D   V   S   D    79

 400 TAT GTG ACC CCA CCC CAG GGC ACC TCT GTC TTT GTC ATC ATC ACC AAA ATG ATC GTT ACT   459
  80  Y   V   T   P   P   Q   G   T   S   V   F   V   I   I   T   K   M   I   V   T    99

 460 GAA AAT CAA ATG CAA GGA TTC TGT CCA GAG AAT GAA GAG AAG TAC CGC TGT GTG TCT GAC   519
 100  E   N   Q   M   Q   G   F   C   P   E   N   E   E   K   Y   R   C   V   S   D   119

 520 AGC CAG TGT GGG CCT GAA CGC TTC CCA GGT GGG GGG ATC CTC ACC GGC CGC TGC GTG AAC   579
 120  S   Q   C   G   P   E   R   F   P   G   G   G   I   L   T   G   R   C   V   N   139

 580 TAC AGC TCT GTT CTC CGG ACC TGT GAG ATC CAG GGC TGG TGC CCC ACT GAG GTG GAC ACC   639
 140  Y   S   S   V   L   R   T   C   E   I   Q   G   W   C   P   T   E   V   D   T   159

 640 GTG GAG ATG CCT ATC ATG ATG GAG GCT GAG AAC TTC ACC ATT TTC ATC AAG AAC AGC ATC   699
 160  V   E   M   P   I   M   M   E   A   E   N   F   T   I   F   I   K   N   S   I   179

 700 CGT TTC CCT CTC TTC AAC TTT GAG AAG GGA AAC CTC CTG CCT AAC CTC ACC GAC AAG GAC   759
 180  R   F   P   L   F   N   F   E   K   G   N   L   L   P   N   L   T   D   K   D   199

 760 ATA AAG AGG TGC CGC TTC CAC CCT GAA AAG GCC CCA TTT TGC CCC ATC TTG AGG GTA GGG   819
 200  I   K   R   C   R   F   H   P   E   K   A   P   F   C   P   I   L   R   V   G   219
```

FIGURE 3 (cont'd)

```
820 GAT GTG GTT AAG TTT GCT GGA CAG GAT TTT GCC AAG CTG GCC CGC ACG GGT GGC GTT CTG  879
220 D   V   V   K   F   A   G   Q   D   F   A   K   L   A   R   T   G   G   V   L    239

880 GGT ATT AAG ATC GGC TGG GTG TGC GAT CTA GAC AAG GCC TGG GAC CAG TGC ATC CCT AAA  939
240 G   I   K   I   G   W   V   C   D   L   D   K   A   W   D   Q   C   I   P   K    259

940 TAT TCC TTC ACT CGG CTG GAT GGA GTT TCT GAG AAA AGC AGT GTT TCC CCT GGC TAC AAC  999
260 Y   S   F   T   R   L   D   G   V   S   E   K   S   S   V   S   P   G   Y   N    279

1000 TTC AGG TTT GCC AAA TAC TAT AAG ATG GAG AAC GGC AGC GAG TAC CGC ACA CTC CTG AAG 1059
280  F   R   F   A   K   Y   Y   K   M   E   N   G   S   E   Y   R   T   L   L   K   299

1060 GCT TTT GGC ATC CGC TTT GAT GTG CTG GTA TAT GGG AAC GCT GGC AAG TTC AAC ATC ATC 1119
300  A   F   G   I   R   F   D   V   L   V   Y   G   N   A   G   K   F   N   I   I   319

1120 CCC ACC ATT ATC AGC TCG GTG GCG GCC TTC ACT TCT GTG GGA GTG GGC ACT GTT CTC TGT 1179
320  P   T   I   I   S   S   V   A   A   F   T   S   V   G   V   G   T   V   L   C   339

1180 GAC ATC ATC CTG CTC AAT TTC CTC AAA GGG GCT GAT CAC TAC AAA GCC AGG AAG TTT GAG 1239
340  D   I   I   L   L   N   F   L   K   G   A   D   H   Y   K   A   R   K   F   E   359

1240 GAG GTG ACT GAG ACA ACA CTG AAG GGT ACT GCG TCA ACC AAC CCA GTG TTC GCC AGT GAC 1299
360  E   V   T   E   T   T   L   K   G   T   A   S   T   N   P   V   F   A   S   D   379

1300 CAG GCC ACT GTG GAG AAG CAG TCT ACA GAC TCA GGG GCC TAT TCT ATT GGT CAC tagggcct 1361
380  Q   A   T   V   E   K   Q   S   T   D   S   G   A   Y   S   I   G   H           397

1362 cttcccagggttccatgctcacccttaggctgcagaacctgcaaacaggccactctatctaagcagtcaggggtgggagg 1441

1442 gggagaagaagggctgctatttctgctgttcaccccaaagactagatccagatatctaggccctcactgttcaacagata 1521

1522 ggcaatgcttcccactaagacttgaatcttgcctttaccccttgcatgcctcccacctgcttccctggatcccaggacag 1601

1602 cagcatccacccctttccaaaggattgagaaaatggtagctaaggttacacccataggacctaccacgtaccaagcactt 1681

1682 ccacacatattatccctttcacccttaaaataatcctataaggtagaaaaaaaaaaaaaaaaaaaaaaaa          1753
```

FIGURE 4

```
   1 gcctccagctgacctctggctcctgtcctctggctccacctgcaccgccctgctcttcctaaggggccaggaagccccca 80

  81 gaagctctaccatcgacgtgggtggtggcacccggctcaccctgagagcagagggcgtgcaggggctcagttctgagcc 160

 161 cagccggcccacc ATG GCA CGG CGG TTC CAG GAG GAG CTG GCC GCC TTC CTC TTC GAG TAT    221
   1              M   A   R   R   F   Q   E   E   L   A   A   F   L   F   E   Y     16

 222 GAC ACC CCC CGC ATG GTG CTG GTG CGT AAT AAG AAG GTG GGC GTT ATC TTC CGA CTG ATC 281
  17 D   T   P   R   M   V   L   V   R   N   K   K   V   G   V   I   F   R   L   I  36

 282 CAG CTG GTG GTC CTG GTC TAC GTC ATC GGG TGG GTG TTT CTC TAT GAG AAG GGC TAC CAG 341
  37 Q   L   V   V   L   V   Y   V   I   G   W   V   F   L   Y   E   K   G   Y   Q  56

 342 ACC TCG AGC GGC CTC ATC AGC AGT GTC TCT GTG AAA CTC AAG GGC CTG GCC GTG ACC CAG 401
  57 T   S   S   G   L   I   S   S   V   S   V   K   L   K   G   L   A   V   T   Q  76

 402 CTC CCT GGC CTC GGC CCC CAG GTC TGG GAT GTG GCT GAC TAC GTC TTC CCA GCC CAG GGG 461
  77 L   P   G   L   G   P   Q   V   W   D   V   A   D   Y   V   F   P   A   Q   G  96

 462 GAC AAC TCC TTC GTG GTC ATG ACC AAT TTC ATC GTG ACC CCG AAG CAG ACT CAA GGC TAC 521
  97 D   N   S   F   V   V   M   T   N   F   I   V   T   P   K   Q   T   Q   G   Y  116

 522 TGC GCA GAG CAC CCA GAA GGG GGC ATA TGC AAG GAA GAC AGT GGC TGT ACC CCT GGG AAG 581
 117 C   A   E   H   P   E   G   G   I   C   K   E   D   S   G   C   T   P   G   K  136

 582 GCC AAG AGG AAG GCC CAA GGC ATC CGC ACG GGC AAG TGT GTG GCC TTC AAC GAC ACT GTG 641
 137 A   K   R   K   A   Q   G   I   R   T   G   K   C   V   A   F   N   D   T   V  156

 642 AAG ACG TGT GAG ATC TTT GGC TGG TGC CCC GTG GAG GTG GAT GAC GAC ATC CCG CGC CCT 701
 157 K   T   C   E   I   F   G   W   C   P   V   E   V   D   D   D   I   P   R   P  176
```

FIGURE 4 (cont'd)

```
702 GCC CTT CTC CGA GAG GCC GAG AAC TTC ACT CTT TTC ATC AAG AAC AGC ATC AGC TTT CCA  761
177 A   L   L   R   E   A   E   N   F   T   L   F   I   K   N   S   I   S   F   P    196

762 CGC TTC AAG GTC AAC AGG CGC AAC CTG GTG GAG GAG GTG AAT GCT GCC CAC ATG AAG ACC  821
197 R   F   K   V   N   R   R   N   L   V   E   E   V   N   A   A   H   M   K   T    216

822 TGC CTC TTT CAC AAG ACC CTG CAC CCC CTG TGC CCA GTC TTC CAG CTT GGC TAC GTG GTG  881
217 C   L   F   H   K   T   L   H   P   L   C   P   V   F   Q   L   G   Y   V   V    236

882 CAA GAG TCA GGC CAG AAC TTC AGC ACC CTG GCT GAG AAG GGT GGA GTG GTT GGC ATC ACC  941
237 Q   E   S   G   Q   N   F   S   T   L   A   E   K   G   G   V   V   G   I   T    256

942 ATC GAC TGG CAC TGT GAC CTG GAC TGG CAC GTA CGG CAC TGC AGA CCC ATC TAT GAG TTC 1001
257 I   D   W   H   C   D   L   D   W   H   V   R   H   C   R   P   I   Y   E   F    276

1002 CAT GGG CTG TAC GAA GAG AAA AAT CTC TCC CCA GGC TTC AAC TTC AGG TTT GCC AGG CAC 1061
 277 H   G   L   Y   E   E   K   N   L   S   P   G   F   N   F   R   F   A   R   H    296

1062 TTT GTG GAG AAC GGG ACC AAC TAC CGT CAC CTC TTC AAG GTG TTT GGG ATT CGC TTT GAC 1121
 297 F   V   E   N   G   T   N   Y   R   H   L   F   K   V   F   G   I   R   F   D    316

1122 ATC CTG GTG GAC GGC AAG GCC GGG AAG TTT GAC ATC ATC CCT ACA ATG ACC ACC ATC GGC 1181
 317 I   L   V   D   G   K   A   G   K   F   D   I   I   P   T   M   T   T   I   G    336

1182 TCT GGA ATT GGC ATC TTT GGG GTG GCC ACA GTT CTC TGT GAC CTG CTG CTG CTT CAC ATC 1241
 337 S   G   I   G   I   F   G   V   A   T   V   L   C   D   L   L   L   L   H   I    356

1242 CTG CCT AAG AGG CAC TAC TAC AAG CAG AAG AAG TTC AAA TAC GCT GAG GAC ATG GGG CCA 1301
 357 L   P   K   R   H   Y   Y   K   Q   K   K   F   K   Y   A   E   D   M   G   P    376

1302 GGG GCG GCT GAG CGT GAC CTC GCA GCT ACC AGC TCC ACC CTG GGC CTG CAG GAG AAC ATG 1361
 377 G   A   A   E   R   D   L   A   A   T   S   S   T   L   G   L   Q   E   N   M    396
```

[GURE 4 (cont'd)

```
1362 AGG ACA TCC TGA tgctcgggccccaactcctgactgggtgcagcgtgaggcttcagcctggagccctggtgggtcc 1437
 397 R   T   S   *                                                                    400

1438 cagccagggcagaggggcctccccaggaagtctcctaccctctcagccaggcagagagcagtttgccagaagctcagggt 1517

1518 gcatagtaggagagacctgtgcaaatctgagctccggctccgaccccacacaccctgagggaggcctaccctagcctcag 1597

1598 ccgctcctggtgggggaatggctgggggttgggcaggaccctcccacacacctgcaccctagcttcgtgcttctctctcc 1677

1678 ggactctcattatccaacccgctgcctccatttctctagatctgtgctctccgatgtggcagtcagtaaccataggtgac 1757

1758 taaattaaactaaaataaaatagaatgaaacacaaaattcaattcctcggctgaactagccacatttcaactgctcagta 1837

1838 gatacgtgtggttagtggctgccatactggacagctcggggcattttcactgtcaaagaaagttctattagacagccctg 1917

1918 cttgagccctgtttcttcctggcttcggtttccctggggaacttatcgacaatgcaagctcctgggcccacccccagacc 1997

1998 tcctgaaccaaaagctccagggctggccgtatgatctgtgtggatggcaaactccccaggccattctgggacctaagttt 2077

2078 aagaagtgccgtcctcgaactttctgactctaagctcctgagcgggagtcagacttagccctgagcctgcacttcctgtt 2157

2158 caggtgcagacactgaacagggtctcaaacaccttcagcatgtgtgttgtgtgctcacgtgccacacagtgtctcatgca 2237

2238 cacaacccagtgtacacaccacctacgtgcacacagcatccttccacactgtgtatgtgaacagcttgggccctgcaaac 2317

2318 acaaccatctacacacatctacacccccaagcacacacacatggtccgtgccatgtcacctccatagggaaaggcttctc 2397

2398 tccaagtgtgccaggccaggacagccctcccagccatgaatccttactcagctacctcgggttggggtgggagccccagc 2477

2478 caaatcctgggctccctgcctgtggctcagccccagctcccaaggcctgcctggctctgtctgaacagaaggtctggggg 2557

2558 aagcgaggggtggagtacaataaagggaatgaggacaaacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 2637

2638 aaaaaa                                                                          2643
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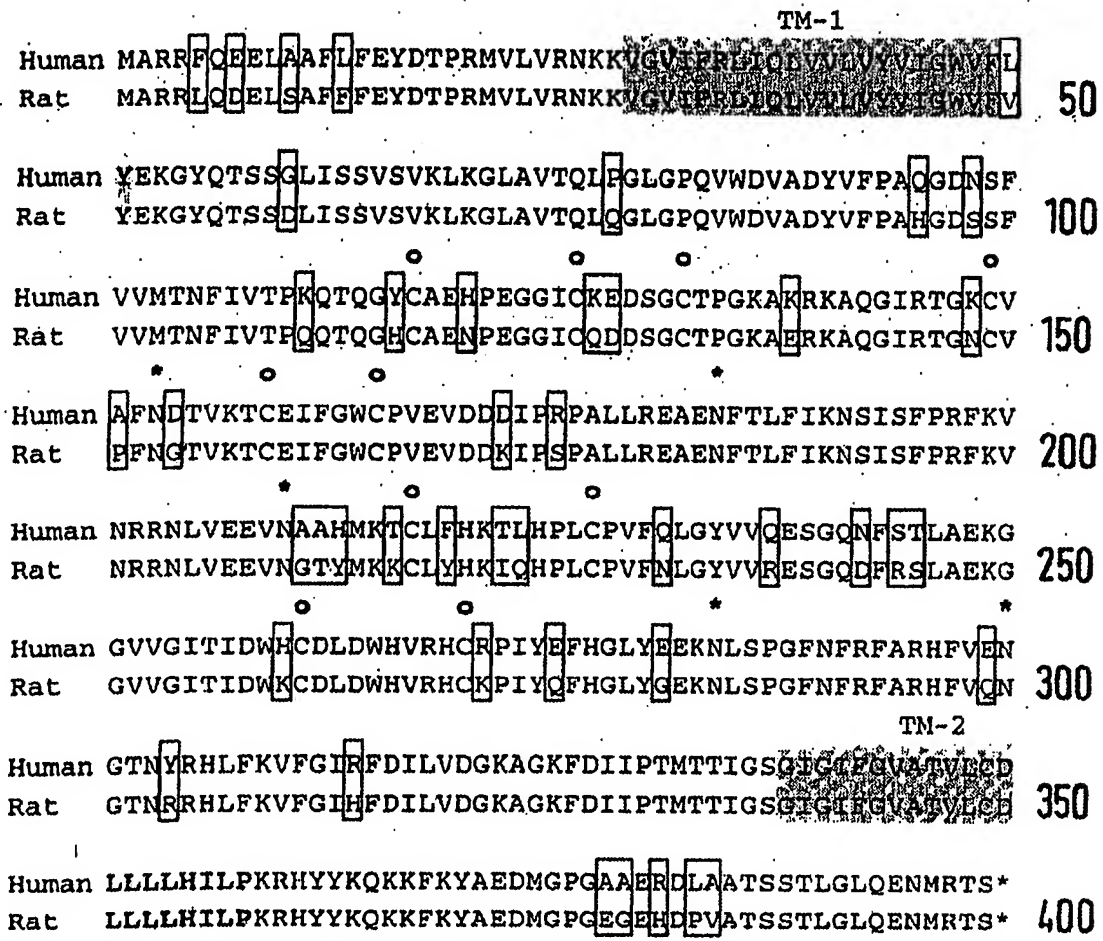


FIGURE 5

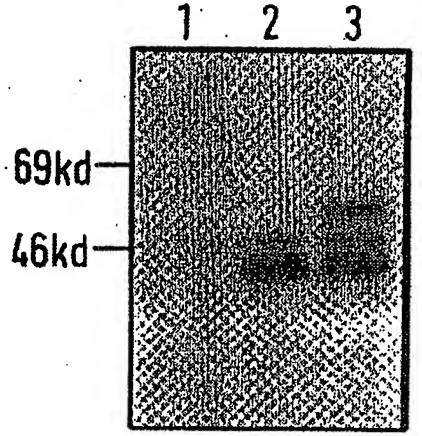


FIGURE 6

PCT/EP95/01968

FIGURE 7

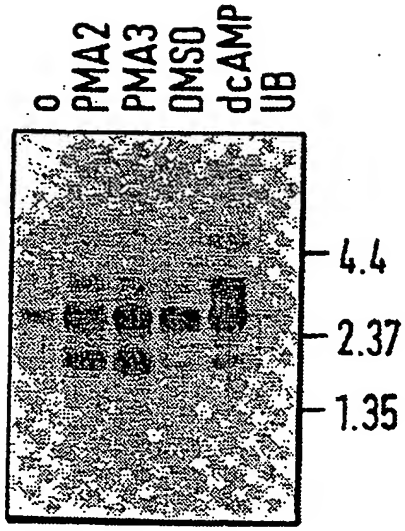
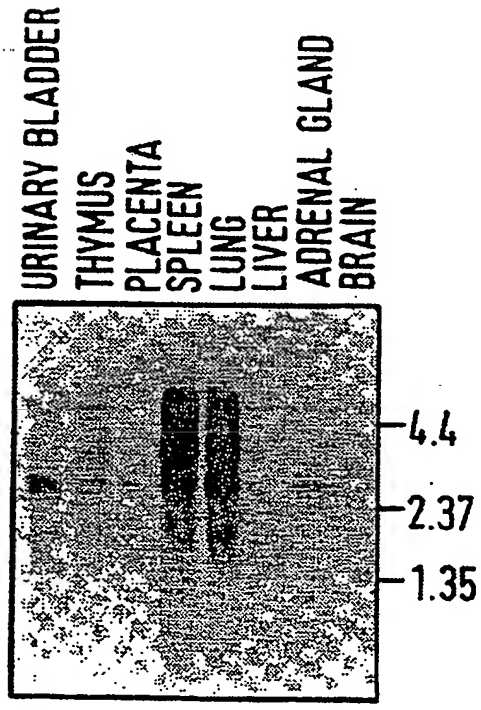


FIGURE 8



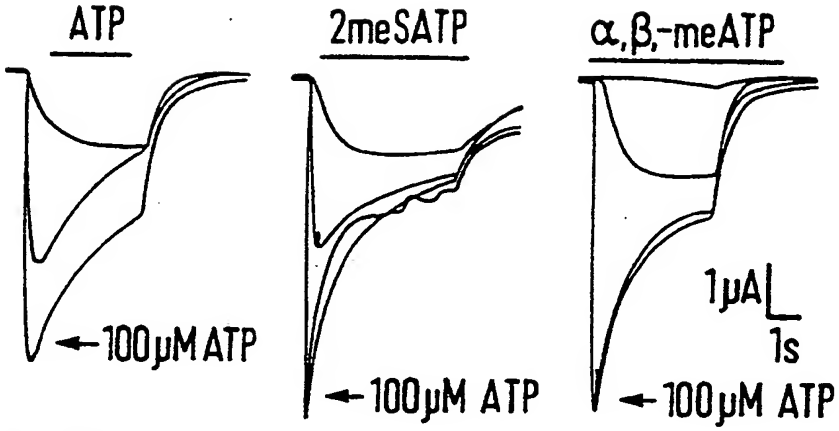


FIGURE 9

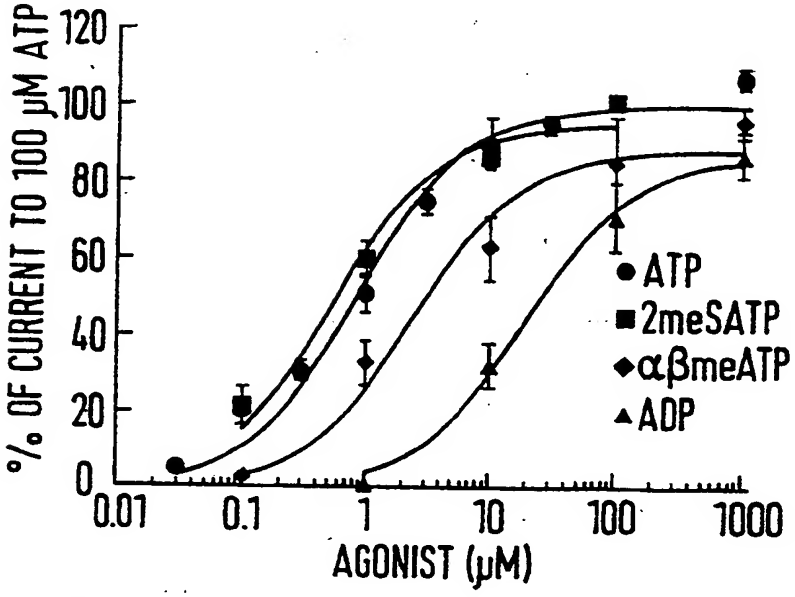


FIGURE 10

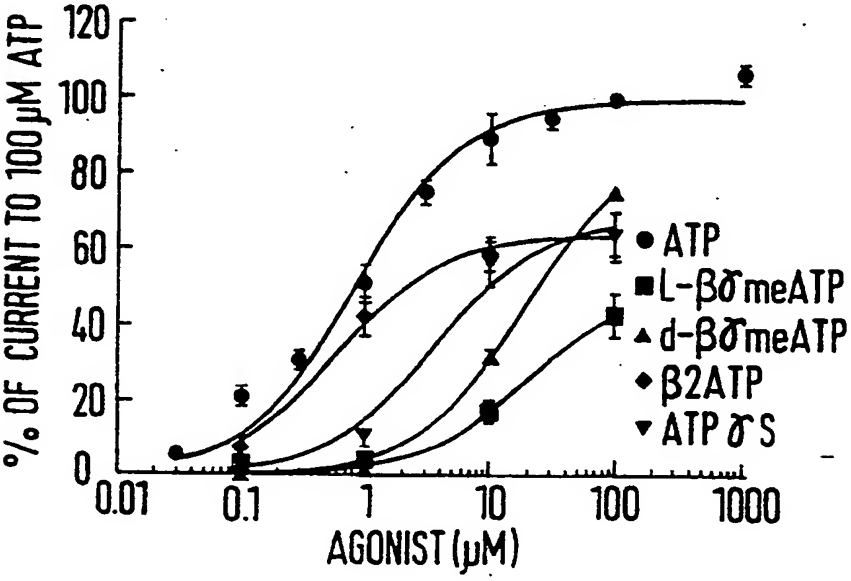


FIGURE 11

FIGURE 12

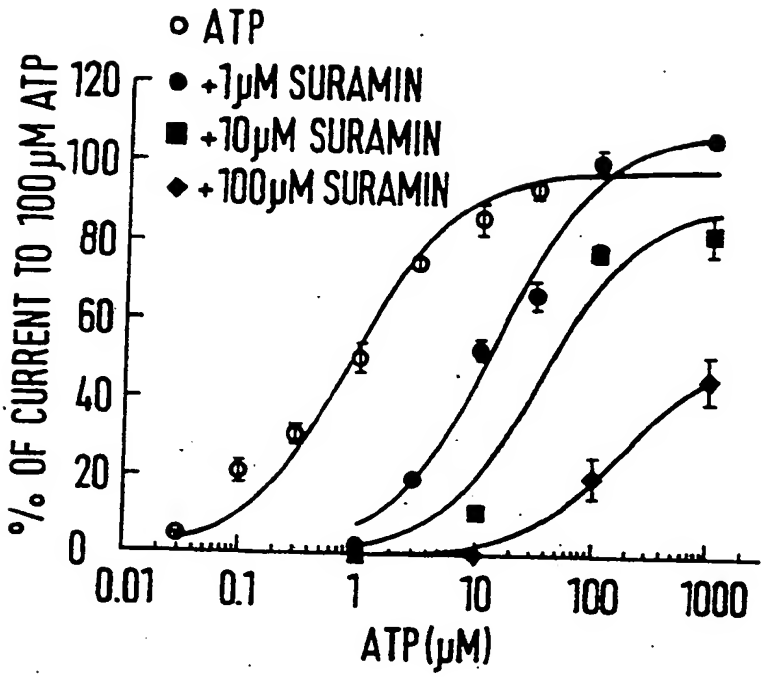


FIGURE 13

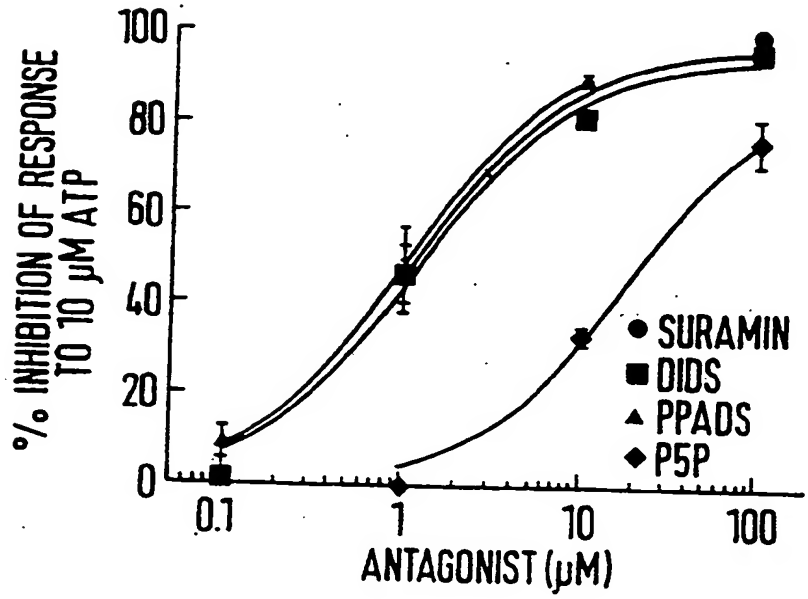
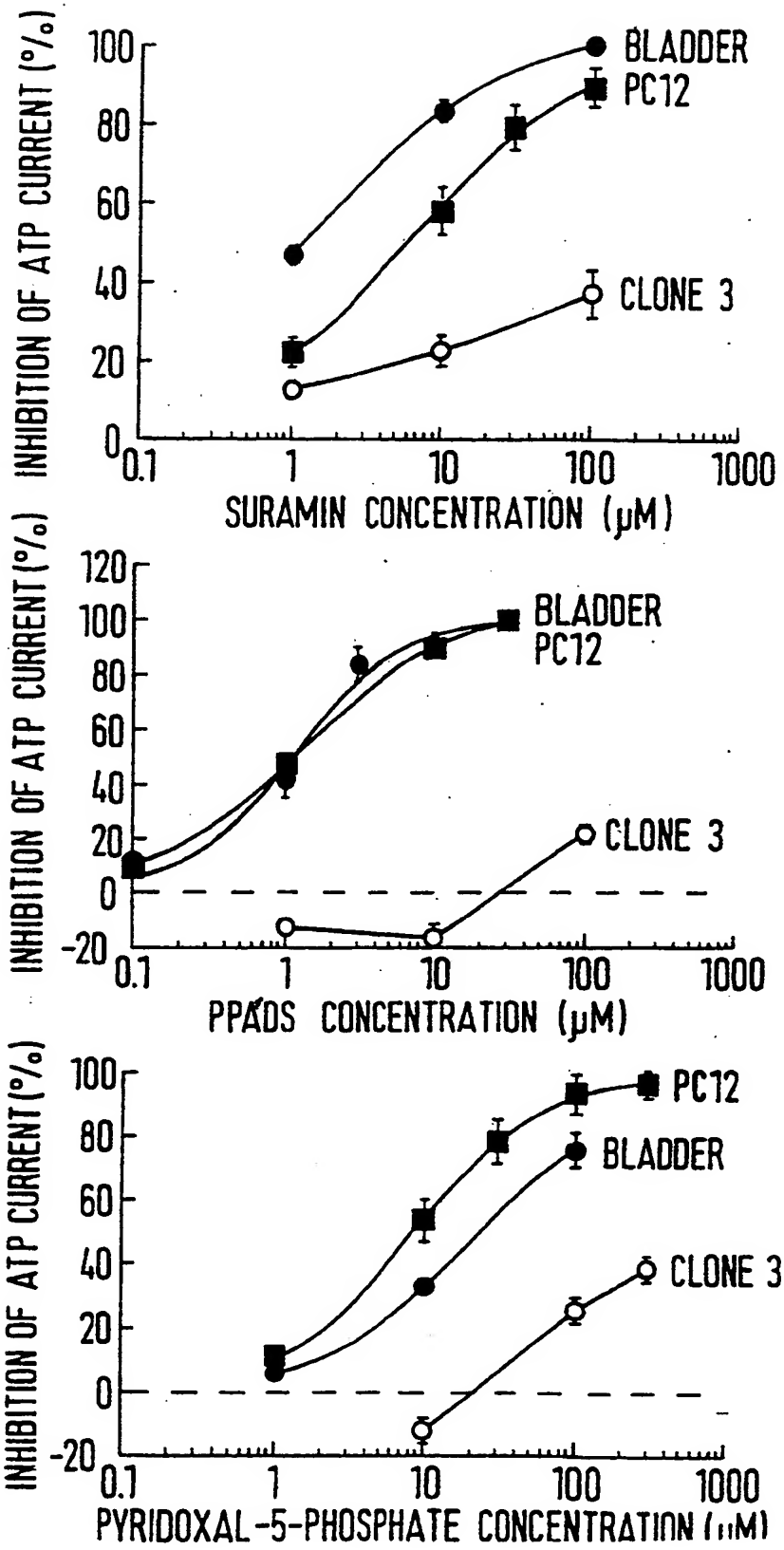


FIGURE 14


INHIBITION OF ATP CURRENT (%)
100
80
60
40
20
0
0.1
1
10
100
1000
SURAMIN CONCENTRATION (μM)
BLADDER
PC12
CLONE 3
INHIBITION OF ATP CURRENT (%)
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
PPADS CONCENTRATION (μM)
BLADDER
PC12
CLONE 3
INHIBITION OF ATP CURRENT (%)
100
80
60
40
20
0
-20
0.1
1
10
100
1000
PYRIDOXAL-5-PHOSPHATE CONCENTRATION (μM)
PC12
BLADDER
CLONE 3

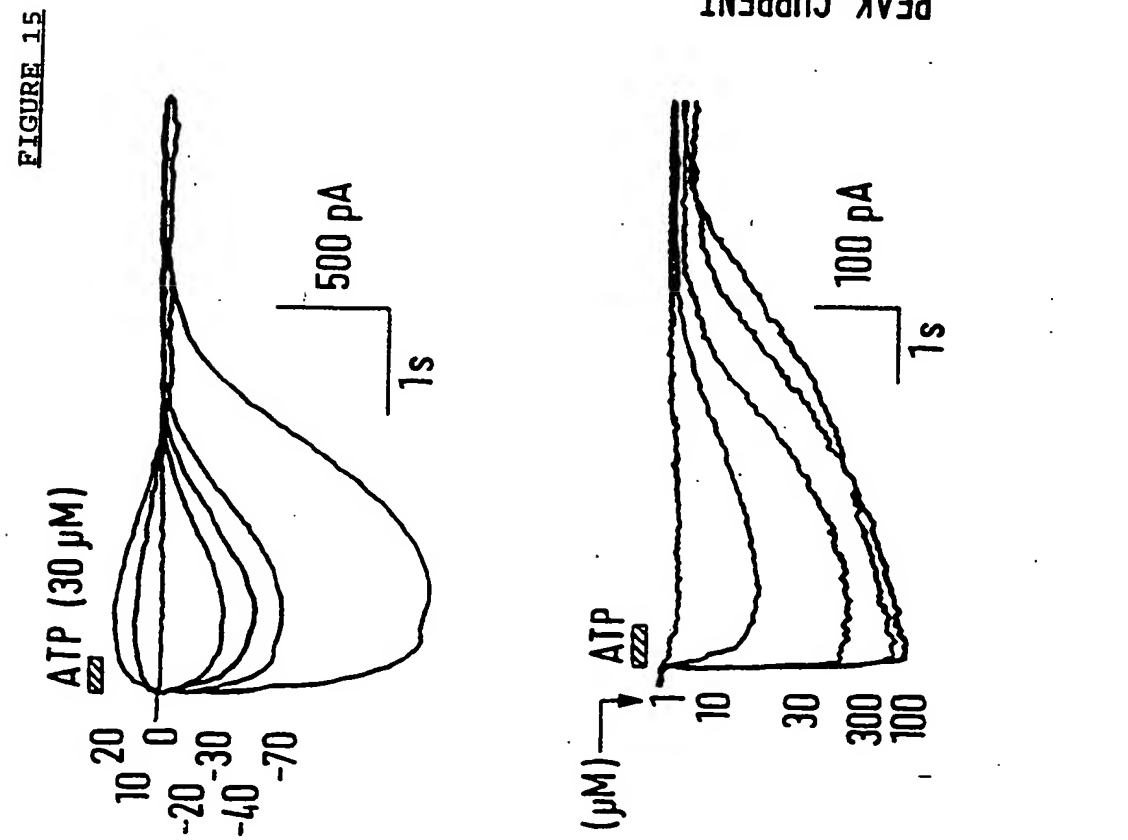

ATP (30 µM)
500 pA
1s
10
20
0
-20
-30
-40
-70
ATP
(µM)
1
10
30
300
100
100 pA
1s
POTENTIAL (mV)
-80
-60
-40
-20
CURRENT (pA)
0.5
-0.5
-1
-1.5
20
40
PEAK CURRENT
(% MAXIMUM)
100
80
60
40
20
0
ATP
α,βmATP
1
10
100
AGONIST CONCENTRATION (µM)

FIGURE 15